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Conservative management of retinoblastoma: Challenging orthodoxy without compromising the state of metastatic grace. “Alive, with good vision and no comorbidity”

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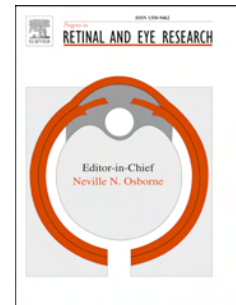
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**Conservative management of retinoblastoma: challenging orthodoxy
without compromising the state of metastatic grace.
“Alive, with good vision and no comorbidity”**

*Dedicated to A. Linn Murphree my paragon and mentor,
and to Akihiro Kaneko who pioneered modern targeted chemotherapy of intraocular
retinoblastoma*

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Abstract

Retinoblastoma is lethal by metastasis if left untreated, so the primary goal of therapy is to preserve life, with ocular survival, visual preservation and quality of life as secondary aims. Historically, enucleation was the first successful therapeutic approach to decrease mortality, followed over 100 years ago by the first eye salvage attempts with radiotherapy. This led to the empiric delineation of a window for conservative management subject to a “state of metastatic grace” never to be violated.

Over the last two decades, conservative management of retinoblastoma witnessed an impressive acceleration of improvements, culminating in two major paradigm shifts in therapeutic strategy. Firstly, the introduction of systemic chemotherapy and focal treatments in the late 1990s enabled radiotherapy to be progressively abandoned. Around 10 years later, the advent of chemotherapy *in situ*, with the capitalization of new routes of targeted drug delivery, namely intra-arterial, intravitreal and now intracameral injections, allowed significant increase in eye preservation rate, definitive eradication of radiotherapy and reduction of systemic chemotherapy.

Here we intend to review the relevant knowledge susceptible to improve the conservative management of retinoblastoma in compliance with the “state of metastatic grace”, with particular attention to (i) reviewing how new imaging modalities impact the frontiers of conservative management, (ii) dissecting retinoblastoma genesis, growth patterns, and intraocular routes of tumor propagation, (iii) assessing major therapeutic changes and trends, (iv) proposing a classification of relapsing retinoblastoma, (v) examining treatable/preventable disease-related or treatment-induced complications, and (vi) appraising new therapeutic targets and concepts, as well as liquid biopsy potentiality.

Key words: retinoblastoma, treatment, intra-arterial chemotherapy, intravitreal chemotherapy, intracameral chemotherapy, complication, metastasis

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1. Introduction

Retinoblastoma is the archetype of developmental tumors and is responsible for 17% of neonatal (Halperin, 2000), 13% of infantile (Vasilatou-Kosmidis, 2003), 6% and 3% of all pediatric cancers under age 5 and 15 years, respectively (Broaddus et al., 2009; Group et al., 2013). Left untreated, this malignancy is rapidly recognizable as a protruding mass. Within the limitations of retrospective diagnosis (Kivela and Polkunen 2003), it is thus not surprising to find retinoblastoma already mentioned in the medical literature at the turn of the XVIth and XVIth centuries by Pawius (Pawius, 1657) and Hildanus (Hildanus, 1682) respectively. According to the Hippocratic theory of four humors still in use at that time, ocular tumors were thought to result from a causal chain, of which the patient's constitution and hygiene was the first link (*causa primitiva*), triggering black bile overflow (*causa antecedens*) pouring into the eye after its transfer to the brain (*causa coniuncta*) (Koelbing, 1954). The advocated treatment was a combination of poultice, leech and purge to eliminate the flood of melancholic humor. This approach was supplanted by enucleation, made possible by the advent of anesthesia in the middle of the XIXth century, leading to the first success in terms of patient survival. This surgical period lasted more than 50 years, until the radiosensitivity of retinoblastoma was discovered by Hilgartner in 1903 (Hilgartner, 1903), allowing for the first time a conservative (globe-preserving) management of the disease. Since then, and for the next 90 years, external beam irradiation became the first line treatment, complemented by focal therapeutic modalities such as cryotherapy, xenon photocoagulation and brachytherapy for less advanced diseased eyes. In 1996, following the recognition of a radio-induced neoplasm predisposition in germline carriers, chemoreduction replaced radiotherapy, which use was relegated to treat chemo-resistant disease in only eyes as an alternative to enucleation. The introduction of this whole-body therapy failed, however, to improve eye preservation in advanced disease, while exposing children to systemic toxicity. This was explained by the pharmacokinetics of the perfused

drugs not reaching tumoricidal concentrations in the various intraocular seeding compartments. To overcome these limitations, the concept of targeted chemotherapy using intra-arterial and intravitreal drug delivery was pioneered in Japan by Akihiro Kaneko already 30 years ago (Kaneko and Takayama J et al, 1990; Yamane et al., 2004). In 2008, the technique of intra-arterial chemotherapy, revisited by Abramson (Abramson et al., 2008), became rapidly adopted and proved its efficacy to control retinal and subretinal tumors, but not vitreous seeds. The latter fully surrendered only to intravitreal chemotherapy, which became available in 2012 following the description of a safety-enhanced injection technique (Munier et al., 2012a). Aqueous seeding, the very last intra-ocular sanctuary to resist virtually all available treatment modalities, became manageable only since 2015 with the description of a technique adapted to safely inject into the anterior and posterior chambers (Munier et al., 2015), now successfully implemented in the current management of aqueous disease (Munier et al., 2017a; Munier et al., 2018). To date, the appropriate combination of all these modalities has enabled unprecedented eye survival rates, even for advanced retinoblastoma, contributing at the same time to the eradication of external beam irradiation in the treatment of intraocular retinoblastoma without jeopardizing patient survival.

2. Epidemiology

Retinoblastoma is a cancer of early childhood, habitually described as a rare tumor. Statistical evidence, however, suggests that it is likely the most common eye cancer worldwide (Kivela, 2009). As a developmental cancer, its incidence is highly dependent on age (Table 1), and therefore difficult to establish. To date, the most stable estimates are derived by assigning incident retinoblastomas to the year the patient was born, a method known as birth cohort analysis (Li et al., 2016; Park et al., 2014; Seregard et al., 2004).

2.1. Incidence

The incidence of retinoblastoma depends on the size of the population and the birth rate (Kivela, 2009). Around 7,980 retinoblastomas are predicted to have been diagnosed in 2015 (Table 2). Predictions for Africa, representing 16% of the world population, are 2,293, or 29% of the total. Almost double the number, 4,258 or 53%, is predicted for Asia, with 60% of the world population. Latin America, North America, and Europe with 8%, 3% and 6% of retinoblastomas also have fewer incident cases relative to their percentage of the world population of 9%, 5% and 10%, respectively. These discrepancies stem from differences in birth rates that range from 33.6 per 1000 population in Africa to 10.5 in Europe (Table 2).

Comprehensive registration of retinoblastoma has succeeded mainly in North America, Europe, and Japan, all of which have low birth rates and easy access to health care. For the rest of the world, registration has been incomplete. Mathematical estimates based on birth rates and population size remain more accurate than published incidences, which are derived from regional rather than national registers and subject to cultural, economic and logistic factors. Published incidence rates have at best approached, and more often fallen short of, those derived mathematically (Li et al., 2016; Usmanov and Kivela, 2014). Registration of childhood cancers in general is incomplete, although coverage increases with advances in diagnosis and treatment (Kroll et al., 2012).

Mathematical estimates are important in order to study and predict trends in incidence of retinoblastoma across countries and regions. Calculations based on World Population Prospects (United Nations, 2017) suggest that the world incidence of retinoblastoma will peak at 8,300 cases in 2045 and then level off (Figure 1). In Asia, Europe, and Latin America, the incidence has been declining, although recent waves of refugees from regions in which birth rates are higher may slow down the decline in Europe. The incidence increases in North America and especially in Africa

where the annual incidence of retinoblastoma is still predicted to rise by 1,300 cases, approaching that of Asia by 2060. Such long-term predictions might be affected by shifts in birth control policies and natural or other disasters such as famine and war, both directly and through refugees and other migrants.

Despite annual variation in crude incidences, especially in small populations and over short observation periods, the incidence of retinoblastoma has remained stable over the last fifty years wherever reliable records exist (Broaddus et al., 2009; Gatta et al., 2012; Gregersen et al., 2016; Li et al., 2016; MacCarthy et al., 2009; Moreno et al., 2014), especially when the analysis is based on birth cohorts rather than on live births or on children of specified age in a given year (Park et al., 2014; Seregard et al., 2004).

Unilateral retinoblastoma represents 61 to 75% of all cases, with 25 to 39% bilateral according to recent population-based series (Andreoli et al., 2017; Azar et al., 2006; Gregersen et al., 2016; Khandekar et al., 2004; MacCarthy et al., 2009; Moreno et al., 2014). Part of this variation is explained by the fact that some initially unilateral hereditary retinoblastomas will progress to bilateral disease (Moreno et al., 2014), while the contribution from familial cases also varies between countries (Usmanov and Kivela, 2014). The incidence of trilateral tumors is estimated to be 3.5% in patients with hereditary disease (de Jong et al., 2015b).

2.2. Prevalence

Data on survivors of retinoblastoma are not readily available (Gatta et al., 2012). In the absence of a national registry, a crude prevalence estimate can be derived as follows: for every child born with retinoblastoma, the number of survivors will be roughly the number corresponding to the average life expectancy in that country or region, deducted by the average retinoblastoma mortality, then multiplied by the average number of retinoblastoma cases per year. To give an example, for a

country with a mean life expectancy at birth of 73 years between 1950 and 2015 (from the World Population Prospects), a mean mortality from retinoblastoma of 3%, and 4 new patients per year, the estimate would be $(73 - (0.03 \times 73)) \times 4$ or 283 survivors.

2.3. *Predisposing factors for sporadic retinoblastoma*

Age. Seven to 10% of retinoblastomas are neonatal (Kivela and Hadjistilianou, 2017), being diagnosed during the first month of life and occasionally at birth. In general, retinoblastoma is diagnosed in children under 3 years of age, is rare after 8 years of age and typically not found after the age of 15 (Gatta et al., 2012; Park et al., 2014; Rangamani et al., 2015). It is virtually unknown in adults (Kaliki et al., 2015a; Sengupta et al., 2016), accounting for less than 0.1% of all retinoblastomas.

Sex. In two large population-based registries of 1,601 and 1,452 patients, the ratio was 1.07 and 1.09 respectively (Andreoli et al., 2017; MacCarthy et al., 2009), while others report a preponderance of boys over girls with a ratio of 1.12 to 1.64 (Li et al., 2016; Moreno et al., 2014; Park et al., 2014; Rangamani et al., 2015), which is higher than the world statistic of 1.06 at birth (United Nations, 2017) (Andreoli et al., 2017; MacCarthy et al., 2009). The reasons for this discrepancy are not known, but it might be that in some cultures boys are preferentially brought to treatment (Li et al., 2016).

Ethnicity and latitude. No consistent difference in the incidence of retinoblastoma by ethnicity or latitude has been reported. However, racial and ethnic disparities in the incidence of retinoblastoma were recently reported in the United States (Friedrich et al., 2017; Steliarova-Foucher et al., 2017), with a significantly higher incidence of bilateral retinoblastoma in the Hispanic population, which remains as yet unexplained.

Parental influence. The risk of sporadic retinoblastoma increases with parental and especially maternal age (Moll et al., 1996; Saremi et al., 2014; Yip et al., 2006), although not consistently (Heck et al., 2012), and with maternal active smoking during pregnancy (Azary et al., 2016).

Precursor lesions. Retinoma has emerged as a frequent intermediary in the genesis of both hereditary and nonhereditary retinoblastoma. In one study, 20% of enucleated eyes with retinoblastoma had a retinoma component in the tumor, generally located towards the tumor base (Eagle, 2009). Like retinoblastomas, retinomas carry two mutated *RBI* alleles, but they will remain quiescent unless additional hits drive them into retinoblastoma (Dimaras et al., 2008b). The apparent high propensity of retinomas to degenerate into retinoblastoma likely explains their rarity as a true benign counterpart of retinoblastoma. It is widely presumed that transformation of long-standing retinomas is responsible for the exceptional adult retinoblastoma (Sengupta et al., 2016).

3. Diagnosis

3.1. Presenting features

Retinoblastoma is the only pediatric cancer presenting with recognizable signs, allowing awareness campaigns to be credited with significant shortening of the time to diagnosis as shown in Switzerland (Wallach et al., 2006). Similarly, the implementation of a national educational program in Honduras allowed to significantly reduce the proportion of patients with extraocular spread at diagnosis within less than a decade (Leander et al., 2007). This unique feature of retinoblastoma has the potential to improve patient and ocular survival by earlier detection, not only in developing countries where more than 90% of retinoblastoma patients reside (see 2), but also in high-income countries where there is still room for increased sensitization.

In high-income countries, it is estimated that less than 3% of retinoblastoma patients are diagnosed with extra-ocular disease at the time of presentation (Chantada et al., 2011). Leukocoria,

a white pupillary reflex, is the inaugural sign in 60% of the cases (Abramson et al., 1998). According to the size and location of the tumor(s) (Fig 2), this sign can be initially intermittent, with detection also influenced by the pupil diameter, and frequently misinterpreted as an optical illusion by the parents or health care providers until the growing tumor elicits a permanent leukocoria. It is not rare to identify leukocoria with flash photography (photoleukocoria), which can precede the diagnosis by several months, especially when the anti-red eye reflex mode of the camera is inactivated, preventing pupil constriction from the pre-photograph flash.

Strabismus occurs when central vision is lost secondary to macular tumoral involvement. It is the second most frequent presenting sign, found in 20% of the cases, manifesting earlier and associated with less advanced disease and better ocular survival than leukocoria (Abramson et al., 1998; Abramson et al., 2003; Shields et al., 2004a; Wallach et al., 2006).

Atypical signs in the remaining 20% of cases usually manifest in older children, where they can masquerade other conditions such as uveitis, glaucoma, cataract, and presumed trauma with intra-ocular hemorrhage. These manifestations in a patient with unsuspected retinoblastoma often lead to invasive diagnostic or curative procedures and are associated with iatrogenic risk of tumor spread. Finally, mid-facial dysmorphic features suggestive of a syndromic form of retinoblastoma with developmental delay and birth defects due to a deletion of the long arm of chromosome 13, can prompt screening of the fundus in otherwise asymptomatic patients (see 5.1.2.2).

In developing countries, the diagnosis can be significantly delayed, with extra-ocular disease present at diagnosis, occurring in less than 10% of cases in middle-income countries (Chantada et al., 2004; Chantada et al., 2011; Kaliki et al., 2019) and up to 45% in low-income countries (Bowman et al., 2008; Waddell et al., 2015). In this context, late presenting signs such as

buphthalmic eyes with neovascular glaucoma, hyphema, non-infective orbital cellulitis and proptosis predominate.

3.2. Clinical examination and ancillary testing

Retinoblastoma is the only pediatric cancer not requiring pathology to confirm the diagnosis, due to its pathognomonic funduscopy features. Indirect ophthalmoscopy with scleral depression under anesthesia is the gold standard for detection of retinoblastoma and is usually sufficient to establish the diagnosis and grouping of eye disease. Fundus photography provides the necessary documentation for monitoring conservative therapy as well as for telemedicine purposes. Ancillary investigations, such as ultrasonography and MRI, are instrumental for the differential diagnosis in eyes with opaque media and for staging purposes, by revealing possible extraocular extension of the disease. During conservative management, the therapeutic strategy is continuously challenged by tumor's response and treatment-related complications. The fine tuning in decision making increasingly relies on ancillary imaging techniques such as fluorescein angiography (FA), optical coherence tomography (OCT) and ultrasonic biomicroscopy (UBM). These imaging modalities have become pivotal in the subclinical detection and monitoring of tumoral and non-tumoral events, and thus have a profound impact on both the treatment strategy and the final clinical outcome. As such, these diagnostic tools should be part of the equipment of any tertiary referral center for retinoblastoma.

3.2.1. Fundus documentation and fluorescein angiography

Iconographic documentation of the fundus is mandatory at presentation and at each new exam, to provide precise and detailed documentation of each tumor comprising location, size and aspect, together with their relation to the optic nerve, macula or ora serrata. Rupture of the internal limiting membrane on the tumor apex, presence of vitreous seeding, retinal detachment (past or present) or

subretinal dissemination are signs of more extensive disease and thus higher risk of recurrence and therapeutic failure. All of these clinical details must be documented accurately to better anticipate the disease course and to adapt the treatment strategy accordingly.

Fundus drawing is the simplest and also the most instructive method of documentation for the examiner, requiring a rigorous sense of observation and faithful interpretation. It is, however, time consuming and examiner-dependent. The schematic representation according to a conventional code may easily fail to depict small changes of the tumor, or slow progression of growth. In addition, exchange with other experts or physicians with this kind of support can be problematic. For these reasons, fundus photography has taken on an important role in the documentation of retinoblastoma, providing objective representation and easier comparison of images. Several models of hand-held digital cameras with corneal contact lenses (30-130°) are commercialized for use in the operating theater, namely the RetCamTM (Clarity Medical Systems, Inc., Pleasanton, CA, USA), the PanoCamTM Pro (Visunex Medical Systems, Fremont, CA, USA), and ICONTM (Phoenix Technology Group, Pleasanton, CA, USA). FA modules are available on all 3 devices. For children over 4 years of age and examined without anesthesia, OptosTM (Marlborough, MA, USA) is an ultrawide-angle imaging system providing a 200° retinal field view in one non-contact capture, even with a pupil diameter as small as 1 mm.

FA of both the anterior and posterior segment plays an essential role in the evaluation of retinoblastoma patients, especially in the context of intra-arterial chemotherapy, by providing undeniable information regarding tumor activity or vascular complications under the following circumstances:

- (i) At presentation, to detect subclinical iris neovascularization (Kim et al., 2014) and assess the tumoral and retinal vascularization status (Ohnishi et al., 1982).

- (ii) After each intra-arterial course, to detect vascular complications such as ischemic retinopathy, associated or not with retinal and/or iris neovascularization (see 7.3.4).
- (iii) At completion of first line and consolidation therapy, to provide a baseline angiogram to be used for future comparison (Shields et al., 1982).
- (iv) In case of suspicion of relapse, especially in the context of type II and III regression patterns, by revealing tumor neovascularization (Ohnishi et al., 1982; Shields et al., 1982).
- (v) In case of new retinal detachment, neovascularization and/or intraocular hemorrhage during conservative treatment, to assess the intraocular vascularization status (Stathopoulos et al., 2018a).
- (vi) To monitor treatment response to intraocular vascular complications such as focal treatment of ischemic retina, retinal detachment repair and/or anti-Vascular Endothelial Growth Factor (VEGF) therapy (Stathopoulos et al., 2018a).

3.2.2. Ultrasonic biomicroscopy (UBM)

UBM yields high resolution images of the anterior segment and structures of the posterior chamber that are hidden from direct visualization, such as the ciliary body, zonules or anterior hyaloid. Over the last decade, UBM has proved to be instrumental in evaluating any anterior extension of retinoblastoma (Moulin et al., 2012; Vasquez et al., 2011). Using a 35 MHz transducer with an acoustic 68 μm axial resolution (OTI Scan 2000; Ophthalmic Technologies, North York, Ontario, Canada), we have shown that tumoral invasion of the posterior chamber can be detected with high sensitivity (81%) and specificity (100%), even in the absence of anterior chamber involvement (Moulin et al., 2012). It is now possible to obtain scans of 12 mm at 50 MHz (AvisoTM, Quantel Medical, Clermont-Ferrand, France) with a 35 μm axial resolution, providing

unprecedented details of the micro-anatomy of the anterior segment (Fig 3) and improvement in terms of tumor detection sensitivity.

In clinical practice, UBM has become a fundamental tool for:

- (i) Assessing the anterior extent of retinoblastoma at diagnosis or at relapse with respect to the integrity of the anterior hyaloid and the anterior uvea (ciliary body and iris) (see 6.2.4).
- (ii) Exploring the extreme periphery and posterior chamber in diffuse anterior retinoblastoma for the identification of the primary tumor, which can be located in the retinal rim under the vitreous base or anterior to the ora serrata (see 6.1.2.3).
- (iii) Identifying a safe pars plana meridian, devoid of parietal tumor growth, as entry site for intravitreal chemotherapy, especially when pupil dilation is compromised or in the presence of opaque media (see 7.2.3).

3.2.3. *Optical coherence tomography (OCT)*

Commercialization of hand-held spectral-domain OCT apparatus (HH-SD OCT), (BioptigenTM, Durham, North Carolina, USA or Optovue iVue, Fremont, CA, USA) specifically adapted for pediatric use, has enabled OCT imaging in children lying in the supine position. The integration of HH-SD OCT in the management of retinoblastoma is attested by an increasing number of publications allowing better understanding and evaluation of both tumoral and non-tumoral abnormalities.

In practice, OCT provides an invaluable tool to improve retinoblastoma care in the following situations:

- (i) Early detection of tumors or tumor growth allowing prompt therapeutic response, particularly beneficial in case of juxta-papillary or juxta-foveal lesions. Specific cases include the following presentations:
 - a. Cryptic retinal tumors (Berry et al., 2016a; Rootman et al., 2013; Seider et al., 2016; Soliman et al., 2017), appearing as round hyperreflective lesions located in the inner or outer retina (Fig 4A, B). Such subclinical findings challenge the classification if present at diagnosis, possibly changing unilateral to bilateral involvement and/or grouping (see 3.3.1).
 - b. Cryptic incipient retinal recurrences (Gaillard et al., 2018) (Fig 4C).
 - c. Epipapillary lesions at a pre-invasive stage (Fabian et al., 2017b) (Fig 4D, E).
 - d. Choroidal invasion (Stathopoulos et al., 2018b) (Fig 4 F).
 - e. Precise delineation of the invisible front line of tumor growth in diffuse infiltrating retinoblastoma (Stathopoulos et al., 2018c) (Fig 9 G-I).
 - f. Cluster of cells (anterior chamber seeding) in the irido-corneal angle and /or placoid growth on the corneal endothelium that could otherwise go unnoticed (anterior chamber OCT) (Fig 13 E).
- (ii) Monitoring of the treatment response in the vitreous (Fig 4G, H) or retina (Fig 4J, K). In the specific case of cryptic type 0 regressed tumors (Saktanasate et al., 2015), OCT enables documentation of a residual hyperreflective lesion that should either receive focal therapy to result in a type 4 regression, or be carefully monitored to prove its inactivity (absence of growth) (Fig 4J, K).
- (iii) Evaluation of the retinal and optic nerve status behind dense retrohyaloid or epiretinal seeding (Hasanreisoglu et al., 2015)(Fig 4 F).

- (iv) Assessment of the foveal integrity to evaluate visual potential. In the specific case of retinal detachment, OCT can be used to monitor foveal detachment while awaiting complete spontaneous or surgical reapplication before intensifying amblyopia treatment (see 7.3.1).
- (v) Documentation of non-tumoral treatment-related alterations such as choroidal thinning post intra-arterial chemotherapy (Maidana et al., 2014) or radio-induced macular edema (Miller et al., 2005).
- (vi) Differential diagnosis of other retinal tumors (Fig 4L-N).

3.2.4. *Magnetic Resonance Imaging (MRI)*

High-resolution contrast-enhanced MRI is currently considered to be the most sensitive technique to evaluate any extraocular extension of retinoblastoma. In middle and high-income countries, it has now completely supplanted computer tomography (CT) imaging, which carries an increased risk of radiation-induced oncogenesis without adding any diagnostic value. The success of MRI in the context of retinoblastoma relies, however, on specific requirements which differ from those used for routine brain and orbit examinations. A consensual standardized protocol for the evaluation of retinoblastoma at diagnosis has recently been proposed by members of the European Retinoblastoma Imaging Collaboration (ERIC) (de Graaf et al., 2012b). In order to achieve high resolution imaging, two techniques using 1.5 or 3 Tesla scanners can be employed. With the 1.5 Tesla scan, the use of a surface coil to increase the signal-to-noise ratio is necessary. Surface coils have, however, the disadvantage of requiring more time for accurate positioning, allow the examination of only one orbit at a time, and are less precise in imaging the posterior part of the orbit due to rapid signal loss. Surface coils are not needed with a 3 Tesla scanner equipped with a 32-channel coil (de Jong et al., 2015a).

MRI plays a critical role in assessing the following:

- (i) Detection of tumor calcifications, which is important in the differential diagnosis especially in cases where the fundus view is obscured. Calcifications can be observed with high sensitivity and specificity on T2 images without the help of CT, as proved by an *ex vivo* MRI study (Rodjan et al., 2015).
- (ii) Patient staging by enabling detection of extra-ocular invasion at the level of the:
 - a. Optic nerve. In the presence of a normal-sized optic nerve sheath, the diagnosis of retrolaminar invasion relies on contrast enhancement after gadolinium injection in T1. However, high resolution-MRI shows limited sensitivity in depicting early-stage optic nerve invasion and therefore cannot substitute for pathological analysis. These limitations are highlighted in a 1.5 Tesla surface coil study with histological verification, which proved to be disappointing in terms of sensitivity, although high specificity (range 0.64-1) and high negative predictive values (range 0.81-0.97) could be confirmed (Brisse et al., 2015). In the case of massive optic nerve invasion, an intra-cranial mass can form and cerebrospinal fluid invasion leading to leptomeningeal enhancement can be visualized.
 - b. Choroid. The normal choroid enhances after gadolinium injection, whereas disruption of the choroid blush is a sign leading to the suspicion of choroidal invasion (de Graaf et al., 2012b).
 - c. Sclera. The sclera does not enhance and is seen as a hypointense line on T2 images. Disruption of this line and the deformation of the globe are signs of scleral invasion.
- (iii) Screening for trilateral retinoblastoma (see 3.6) (Fig 5).

- (iv) Diagnosis of second primary neoplasms, especially in hereditary retinoblastoma survivors treated by external beam irradiation (see 3.8).

3.3. Classifications

Classification schemes in cancer aim to provide a universal language for the evaluation of the disease extent, which is necessary to determine and compare different treatment strategies and to enable prognostication. Unlike cancers affecting other organs, where tumor excision or biopsy is usually performed for diagnosis confirmation and histopathologic grading, the diagnosis of retinoblastoma is clinical. Classifying retinoblastoma poses, however, a particular challenge as both intraocular and extraocular tumor extension need to be assessed in order to predict not only the likelihood of eye preservation, but also the risk of dissemination, which in turn correlates with patient survival.

Historically, the classification of retinoblastoma was divided into multiple intraocular retinoblastoma eye grouping (see 3.3.1) and patient staging (see 3.3.2) systems, still variously used and therefore making comparison between studies difficult. The 8th edition of the American Joint Committee on Cancer “Tumor, Node, Metastasis and Heritability” (TNMH) classification has recently been proposed to more accurately predict the salvage of the eye(s), metastasis and patient survival and holds the promise to provide a single universal classification (Mallipatna et al., 2017)(Table 3).

Surprisingly, despite the broad use of OCT in retinoblastoma patients (see 3.2.3), none of the existing classification systems have been adapted to consider possible subclinical findings at diagnosis. Moreover, although heavily-pretreated eyes are being increasingly offered salvage therapies, a classification of intraocular retinoblastoma at relapse is still missing and precludes the possibility to compare study outcomes of salvage treatments. This prompted us to add subclinical

features at diagnosis in the TNMH classification system (see 3.3.1) as well as to propose a classification at relapse (see 3.3.3).

3.3.1. Grouping of the eye at presentation (RE, IIRC, ICRB; COG; TNMH)

A system to evaluate the intraocular extent of the disease was first introduced in the 1960s by Reese and Ellsworth, who stratified intraocular retinoblastoma into five groups (RE I to V), predicting eye survival in response to external beam radiotherapy (Reese and Ellsworth, 1963). The therapeutic shift from external beam radiotherapy to chemoreduction motivated the introduction by Linn Murphree, in 2005, of a novel International Intraocular Retinoblastoma Classification (IIRC), recapitulating the natural history of retinoblastoma into five groups (A to E) (Table 4) (Linn Murphree, 2005), with the presence of group E features being an absolute indication for enucleation. In 2006, this classification was revisited with some modifications by the group of Philadelphia (Shields et al., 2006a), followed in 2011 by the Children's Oncology Group (COG) giving rise to the International Classification of Retinoblastoma (ICRB) and the COG, respectively. At the present time, the parallel use of 3 coexisting A to E classifications confuses the literature, especially regarding advanced eyes and impairs comparison of different clinical reports (Scelfo et al., 2017). For example, in a study of more than 2000 eyes (Kaliki et al., 2019), up to 56% of ICRB group E eyes would have been classified IIRC group D eyes, since IIRC group D eyes with tumor occupying 2/3 of the eye volume are reclassified ICRB group E eyes.

Although the TNMH classification includes the use of radiologic imaging to assess any optic nerve involvement (Mallipatna et al., 2017) (Table 3), it fails, like the previous ones, to allow grouping of invisible tumors only detected by OCT, as may be the case in the context of screening *RB1* mutant carriers with a positive family history. We therefore propose to revise the TNMH classification by adding categories scT1a and b for subclinical staging: scT1a in case of subclinical

tumor(s) at least further than 1.5 mm from the optic nerve head or 3 mm from fovea, and scT1b if closer (Fig 4A).

3.3.2. Patient staging at presentation (IRSS, TNMH)

The first classification system for retinoblastoma aiming to cover the whole spectrum of the disease (IRSS) was introduced in 2006 by a multidisciplinary team of physicians from different countries and based on clinical evaluation, imaging and histopathology (Chantada et al., 2006a) (Table 5). Two years later, the same group standardized the processing of the enucleated eyes in order to obtain reliable histopathologic results and fresh tumor for molecular testing to define pathological risk factors for extraocular relapse (Sastre et al., 2009). The recently introduced TNMH classification considers not only intraocular, extraocular and pathological disease but also, for the first time, the hereditary status and aims to become the gold standard reference in the future (Mallipatna et al., 2017).

The recommended procedures for assessing extraocular dissemination in retinoblastoma include contrast-enhanced MRI of the brain and orbits (de Graaf et al., 2012b) as well as bone marrow and cerebrospinal fluid cytopathologic evaluation, with or without PCR-based detection of specific molecular biomarkers (Laurent et al., 2016) (see 3.7.1).

Staging investigations, such as lumbar puncture and bone marrow aspiration/biopsy are relatively invasive and require general anesthesia. They are not commonly performed in early stages (IRSS stage 0 or 1) where the risk of harboring disseminated disease is considered minimal. These investigations, as well as skeletal scintigraphy are, however, indicated in case of symptoms (pain, neurologic manifestations), exteriorization of the disease or high-risk pathology features reported after enucleation (Azar et al., 2003; Barai et al., 2004; Karcioğlu et al., 1997; Moscinski et al., 1996; Pratt et al., 1989). If conservative treatment is attempted, some groups advocate cytological

evaluation of the cerebrospinal fluid in cases where the optic nerve cannot be visualized (Mallipatna et al., 2017). At the present time, however, a universal consensus especially for the metastatic work-up concerning conservatively-treated advanced intraocular disease is still missing. The development of more precise or less invasive techniques, such as molecular biomarkers in cerebrospinal fluid and bone marrow (Torbidoni et al., 2015a), or functional imaging, would be ideal for diagnosis and follow-up of disseminated disease (Kohe et al., 2015).

3.3.3. Classification of retinoblastoma relapse (RSU classification)

Similar to the classification of retinoblastoma at presentation, the classification of retinoblastoma at relapse aims to standardize appropriate treatment for relapse, achieve a better comprehension of treatment outcomes, predict prognosis for ocular salvage and define enucleation criteria for secondary enucleation. However, because recurrences do not necessarily involve the retina (R), but can be exclusively extraretinal with isolated seeding (S) or uveal involvement (U), it is not possible to classify them by applying one of the grouping systems used at diagnosis. Therefore, we propose a specific classification for relapsing retinoblastoma based on its localization which is detailed in Table 6. In this classification, the recognition of tumor relapse does not only rely on clinical observation but also on imaging devices such as UBM (Chhablani et al., 2010; Stathopoulos et al., 2018b) or SD-OCT (Fabian et al., 2017b; Gaillard et al., 2018). The definition of relapse or recurrence refers to any tumor regrowth or new growth occurring after a minimum of 2 months progression-free and treatment-free follow-up at a monthly examination. In contrast, persistent, due to absence of response, or progressive disease despite ongoing treatments, as well as new primary retinal tumors, have to be distinguished from recurrence, and as such are not concerned by the proposed RSU-classification. Moreover, caution should be taken not to label as relapse, active

disease diagnosed without an intermediate progression-free follow-up interval of at least two months since the last treatment, as in such cases disease persistence/progression cannot be excluded. Prompt secondary enucleation is mandatory for relapse in the presence of persisting vitreous hemorrhage obscuring the fundus, neovascular glaucoma, phthisis bulbi or suspicion of extra-ocular extension.

3.4. Differential diagnosis

The list of conditions simulating retinoblastoma includes a broad spectrum of both acquired and congenital diseases (Balmer and Munier, 2007; Shields et al., 2013) (Table 7). In children seen in non-ocular oncology specialized centers, leukocoria, the main presenting sign of retinoblastoma, was due to congenital cataract in 25-65% of the cases and retinoblastoma in only 6-18% (Haider et al., 2008; Meier et al., 2006). In a retrospective review of nearly 2,800 cases specifically referred for suspected retinoblastoma in a tertiary center in the United States of America, 78% had a confirmed retinoblastoma whereas 22% suffered a different disease, dominated by Coats' disease (Shields et al., 2013). The latter has notably the particularity of mimicking retinoblastoma at different stages, including endophytic macular retinoblastoma group B in Coats' disease stage 2B2, characterized by the presence of a subfoveal pseudo-tumor measuring up to 2.5 mm and sometimes associated with pseudo-feeding vessels (Daruich et al., 2017), or exophytic retinoblastoma group D and E in Coats' disease stages 3B and 4, respectively (Gaillard et al., 2014). In the majority of cases, a comprehensive history with thorough clinical examination and appropriate ancillary tests is usually sufficient to avoid misdiagnosis and undesirable treatments (Shields et al., 2013). The indication for fine needle biopsy should be limited to extraordinarily unusual circumstances (Karcioglu et al., 2002).

3.5. Prenatal screening for retinoblastoma

Early screening for retinoblastoma is advised for children with a positive family history of the disease. Intra-uterine and early postnatal retinoblastoma lesions are predominantly located in the posterior pole, where they are more likely to compromise vision (Kivela and Hadjistilianou, 2017; Soliman et al., 2016) and known for their rapid growth with an estimated doubling time of 2 weeks (Shah et al., 2010).

Before genetic testing and the use of modern imaging techniques (high frequency ultrasound probes and MRI), diagnostic screening for retinoblastoma was performed postnatally with indirect ophthalmoscopy (Abramson et al., 2002). With the introduction of *RB1* mutational testing, offspring of parents with a known mutation can now be screened during pregnancy. Currently, DNA extraction for mutational analysis can be proposed either by chorionic villus sampling at the end of the first trimester (11 weeks of gestation) or by amniocentesis during the second trimester (16 weeks of gestation). Parents should be informed of a procedure-related potential miscarriage risk of 0.22% and 0.11% after chorionic villus sampling and amniocentesis, respectively (Akolekar et al., 2015). Confirmation of a fetal *RB1* mutation allows parents to make an informed decision as to whether or not to proceed with the pregnancy. Standards for the follow-up of a fetus with high-risk to develop the disease do not currently exist. In Lausanne, for parents who decide to proceed with the pregnancy despite confirmation of a fetal *RB1* mutation, or for those who refused amniocentesis, fetal ultrasonography is performed at 26 weeks of gestation and repeated every 3 weeks until 32 weeks, and then every 2 weeks until 36 weeks of gestation. The ultrasonography includes standard 2D and 3D images of fetal anatomy acquired through a magnified axial view of both eyes (Fig 6). The 3D acquisition of volume is an integral part of our evaluation as it allows systematic analysis of the posterior wall of the eye. An

alternative imaging detection method is fetal MRI, which, compared to ultrasonography, has a higher contrast resolution but is more affected by fetal motion (Staffieri et al., 2015). In case of tumor detection, a consultation with an ocular oncologist, perinatologist and neonatologist is needed to balance the burden of an early term delivery, preceded or not by pulmonary maturation therapy, against the oncologic risks of management of a full-term delivery.

In practice, only few cases of fetal retinoblastoma have been published. Two were very large lesions that deformed not only the globe but also the face (Maat-Kievit et al., 1993; Salim et al., 1998). Five occurrences of small intraocular retinoblastomas were reported either by ultrasonography (Paquette et al., 2012; Soliman et al., 2016; Stathopoulos et al., 2018d; Toi et al., 2003) or by MRI (Staffieri et al., 2015). Ultrasonography is not sensitive enough to detect tumors measuring less than 2 mm in thickness. In a fetus carrying a *RBI* mutation, two lesions of 1.2 and 1.9 mm, respectively, were detected at the posterior pole in each eye by MRI performed at 35 weeks of gestation (Staffieri et al., 2015). In a prospective study of the use of both USG and MRI in 6 high risk fetuses, fetal MRI did not detect any tumors, whereas one could be seen on USG, possibly because of the limitation of slice thickness (Paquette et al., 2012).

A recent retrospective observational study suggested that induced early delivery (by 37 weeks of gestation), which remains a controversial procedure (Gombos, 2012), might benefit the child carrying a mutated *RBI* in terms of treatment-related morbidity and ultimate salvage of vision (Soliman et al., 2016). Noteworthy, when retinoblastoma is diagnosed during the neonatal period, at least half of the children initially have a unilateral rather than bilateral tumor, typically group B, but almost all germline mutation carriers later progress to bilateral involvement (Kivela and Hadjistilianou, 2017; Soliman et al., 2016). On the other hand, all infants with neonatal retinoblastoma have at least one eye with a tumor close to the foveola, whereas the macula of the

fellow eye is often spared and loss of reading vision from both eyes is consequently infrequent (Imhof et al., 2006; Kivela and Hadjistilianou, 2017; Soliman et al., 2016).

3.6. Trilateral retinoblastoma

Approximately 3.5% of patients with hereditary retinoblastoma will develop an intracranial tumor, located most often in the pineal gland and less frequently in the supra- or parasellar region (de Jong et al., 2014a) (Fig 5A, B). Such a primitive neuroectodermal tumor associated with hereditary retinoblastoma was first described in 1977 (Jakobiec et al., 1977) and in 1982, named trilateral retinoblastoma (Bader et al., 1982). Originally considered to have a dismal prognosis (Kivela, 1999; Paulino, 1999), sophisticated imaging allowing earlier detection and improved chemotherapy regimens have, since 1995, significantly increased 5-year survival rates from 6% to 44% for pineal tumors and from 0 to 57% for non-pineal tumors (de Jong et al., 2014a). On the other hand, understanding that children frequently have a cystic, non-neoplastic pineal gland with radiological features that differ from pineoblastoma, has helped to avoid over-diagnosis of trilateral retinoblastomas (Barboriak et al., 2001a; Beck Popovic et al., 2006).

3.6.1. Incidence, early detection and screening

The risk period of developing trilateral retinoblastoma extends from birth to 7 years of age (de Jong et al., 2014a; Kivela, 1999), with 95% of the cases diagnosed before the age of 5 (de Jong et al., 2015b). Patients with hereditary retinoblastoma have a higher risk of developing trilateral disease compared to those with non-hereditary disease, the latter carrying the same risk as the normal population. A recent meta-analysis restricted to cohorts with at least 100 retinoblastoma patients estimated the risk of developing trilateral disease to be 3.8% in bilateral disease (2.9% for pineal tumors) and 3.5% (3.2% for pineal tumors) when considering all hereditary retinoblastoma,

including unilateral cases with a positive family history (de Jong et al., 2015b). This naturally leads to the question of the need and the frequency at which one should screen for trilateral retinoblastoma with MRI.

Trilateral retinoblastomas detected synchronously with intraocular retinoblastoma are usually smaller in size, asymptomatic and have a better prognosis compared to those detected metachronously (de Jong et al., 2014a; Kivela, 1999). Five-year survival is also more favorable if the trilateral tumor is pineal and less than 15 mm in size (de Jong et al., 2014a). Baseline screening is therefore currently considered justified by most authors (De Ioris et al., 2014; de Jong et al., 2014a; Rodjan et al., 2012), but cost-effectiveness of subsequent imaging remains controversial (Moll et al., 2002).

3.6.2. Influence of the retinoblastoma treatment on the incidence of trilateral retinoblastoma

Previous radiotherapy for retinoblastoma, especially before the age of 12 months, has been associated with a potentially higher incidence of pineal trilateral retinoblastoma in patients with hereditary retinoblastoma, even though the pineal gland is usually not (directly) within the field of radiation (Moll et al., 2002). The question of whether previous systemic chemotherapy is protective of developing trilateral retinoblastoma is still debated with reports that favor (Shields et al., 2001) or challenge such a preventive effect (Chantada et al., 2014). In the current era of intra-arterial and intravitreal chemotherapy, the likelihood to develop trilateral retinoblastoma does not seem to have increased, with a reported incidence of 2.7% at 5 years follow-up (Habib et al., 2018).

3.6.3. Improved treatment of trilateral retinoblastoma: high-dose chemotherapy

Trilateral retinoblastoma used to be treated with surgery, chemotherapy, and radiotherapy (Blach et al., 1994; Jubran et al., 2004). In 2010, a multimodality approach to metastatic retinoblastoma with intensive induction of chemotherapy, high-dose chemotherapy and stem cell reinfusion was

reported to provide a 5-year survival of 67% in a small series of patients with non-central nervous system (CNS) metastases (Dunkel et al., 2010c) and a cure for only 1 out of 6 patients with CNS dissemination from trilateral retinoblastoma (Dunkel et al., 2010b). This led to a collaborative, prospective clinical trial for metastatic or trilateral retinoblastoma by the Children's Oncology Group (COG) and Grupo de America Latina de Oncologia Pediatrica (GALOP). The treatment consisted of four courses of intensive chemotherapy and, according to response, a three-drug high-dose chemotherapy regimen with stem cell reinfusion, with or without radiotherapy. The results recently confirmed a 3-year event-free survival of 79% after non-CNS metastases, whereas the survival rate remained at an unsatisfactory 8% after CNS metastases or trilateral retinoblastoma (Dunkel et al., 2017).

3.6.4. Differential diagnosis: pineal cysts

Technological progress in imaging has contributed to an increased observation of pineal cysts, a benign finding reported in more than half of the pediatric population using 3 Tesla MRI (Whitehead et al., 2013a). These lesions must be distinguished from trilateral retinoblastomas in order to avoid the administration of unnecessary chemotherapy. On 3 Tesla MRI, typical benign cysts are characterized by an enlarged pineal gland with a hypointense central region on T1-weighted images, isointense on T2-weighted images with a thin wall that enhances after gadolinium injection, and no nodular structures (Barboriak et al., 2001a; Whitehead et al., 2013b). They can increase in size during the first year of observation as the child grows, and then stabilize or decrease in size (Fig 5 E-G) until the age of 4-5 years (Sirin et al., 2016). Pineal cysts in retinoblastoma patients have a reported incidence ranging between 5-37%, with some authors indicating an association with hereditary disease (Beck Popovic et al., 2006; Ruiz Del Rio et al., 2014), whereas others do not (Ramasubramanian et al., 2013). They are comparable in size with cysts in age-

matched healthy controls (Pham et al., 2015), and do not progress to a tumor (Barboriak et al., 2001b; Beck Popovic et al., 2006; Karatza et al., 2006; Ramasubramanian et al., 2013).

Contrary to typical pineal cysts, nodular pineal cysts can transform to pineoblastoma (de Jong et al., 2014b) (Fig 5 C, D). Moreover, in some instances pineoblastomas can also show a cystic part. Any unusual feature in a pineal cyst calls therefore for close follow-up (Rodjan et al., 2012). A retrospective analysis (de Graaf et al., 2012a) by the European Retinoblastoma Imaging Collaboration (ERIC) recently resulted in a description, terminology and classification that allows clinicians to distinguish benign pineal cysts from those that need to be followed because of risk of transformation to pineoblastoma (Sirin et al., 2016).

3.7. *Metastatic retinoblastoma*

Metastatic retinoblastoma at presentation is rare in high-income countries, but represents the most frequent cause of death for this tumor worldwide (Canturk et al., 2010). In high-income countries, the few cases seen are those developing metastasis after initial treatment with enucleation or conservative therapy. The median time between diagnosis of intraocular retinoblastoma primarily enucleated with high pathologic risk factors and first evidence of metastases is 10 months (range: 2-24 months) in patients receiving adjuvant chemotherapy, but appears to occur earlier at 5 months in untreated ones (Antoneli et al., 2003; Chantada et al., 2010; Cozza et al., 2009; Lu et al., 2018; MacKay et al., 1984; Rodriguez-Galindo et al., 2003). In low-income countries, metastatic dissemination is generally already observed at diagnosis in older children who present with extensive orbital masses or after parents declined enucleation of an eye with intraocular disease (Canturk et al., 2010), and is attributed to delayed diagnosis/treatment rather than a difference in tumor biology (Chawla et al., 2016; Leal-Leal et al., 2006).

3.7.1. *Diagnosis of metastatic retinoblastoma*

Retinoblastoma may metastasize to the CNS, bone, bone marrow or lymph nodes and, rarely, to other sites such as the liver. The diagnosis of metastatic disease can occur in asymptomatic patients during a work-up motivated by clinical features at presentation, presence of classic high-risk pathologic findings (Sastre et al., 2009), or possibly by the degree of anaplasia (Mendoza et al., 2015) after enucleation, or in symptomatic patients during/after conservative treatment. In most cases of metastatic disease at diagnosis, massive orbital extension occurs along with distant metastatic dissemination (Kaliki et al., 2017). The detection of minimally disseminated disease in bone marrow and cerebro-spinal fluid at diagnosis and follow-up can be now evaluated by means of quantitative polymerase chain reaction of cone-rod homeobox (CRX) transcription factor messenger RNA (Laurent et al., 2016).

CNS metastasis. CNS involvement occurs mainly by direct extension of the tumor via the optic nerve or the sub-arachnoidal space (leptomeningeal dissemination), but can also be parenchymal or paraspinal through hematogenous dissemination, and rarely as a direct CNS extension through a facial bone. In the case of trilateral retinoblastoma, the tumor may also disseminate to the cerebrospinal fluid from a midline mass (Popovic et al., 2007), typically resulting in a leptomeningeal dissemination (Laurent et al., 2013). Interestingly, molecular studies using polymerase chain reaction for detecting minimally disseminated disease showed that cerebrospinal fluid relapses occurred more commonly as an isolated event with negative bone marrow, suggesting that the route of dissemination to the cerebrospinal fluid in retinoblastoma is mainly through the optic nerve and independent of systemic dissemination (Torbidoni et al., 2015b) or due to trilateral seeding (Torbidoni et al., 2018). However, seeding by subclones arising from the systemic

circulation at diagnosis, which persists during therapy, cannot be ruled out. Affected subjects may show increased intracranial pressure resulting in headache, irritability, vomiting, vision loss, and less commonly, focal neurological signs. Clinical examination includes evaluation of the orbit and facial bones as well as evaluation of the whole neuroaxis. CNS dissemination is investigated by both cerebrospinal fluid cytology (Bakhshi et al., 2011) and imaging, with the latter being performed prior to diagnostic lumbar puncture in order to exclude intracranial hypertension. A cytopsin of cerebrospinal fluid cytology typically shows retinoblastoma cells in clusters. If isolated, these cells can, however, be missed or confused with normal lymphocytes in young children. For this reason, the cytopsin should always be examined microscopically, and malignant cells confirmed with immunocytology for GD2 or CRX. If this procedure is not available, flow cytometry examination, usually available in centers that also treat pediatric leukemia, can be useful to depict the non-hematopoietic origin of the cells by showing the lack of expression of molecules such as CD45 antigen with positivity for CD56 (Shen et al., 2013).

Bone and bone marrow metastasis. Bone metastasis may present as a painful and palpable mass in the long bones, but facial bones may also be affected, as well as the diploe of the neurocranium. Bone marrow invasion may occasionally be suspected by detecting abnormal blood counts, especially thrombocytopenia, but is more commonly detected in asymptomatic patients by bone marrow biopsy or on imaging such as MRI or positron-emission tomography (Chantada et al., 2006b). Bone marrow evaluation needs at least 2 aspirates and 2 biopsies and, if available, immunocytology using markers like GD2 (Chantada et al., 2006b) or reverse transcriptase quantitative PCR for CRX mRNA should be performed (Torbidoni et al., 2015a).

3.7.2. Treatment of metastatic retinoblastoma

Although retinoblastoma is a chemosensitive tumor, metastatic disease is not curable with conventional treatment and is therefore considered to have a poor prognosis, especially in cases of CNS involvement (Kaliki et al., 2017; Pant et al., 2017). High dose chemotherapy, followed by autologous stem cell rescue with radiation to the sites of bulky disease, has been the only treatment reported to date to potentially cure these patients (Dunkel et al., 2010c; Palma et al., 2012), allowing remission in close to 70% of children in a series of 15 patients with metastatic retinoblastoma not involving the CNS (Dunkel et al., 2010c), whereas those with CNS involvement have only anecdotally benefitted from it (Dunkel et al., 2010a). Focusing on intrathecal chemotherapy either with topotecan (Laurie et al., 2005) or radio-immunotherapy with radiolabeled anti-GD2 antibody (Kramer et al., 2010) may improve the currently dismal outcome.

3.7.3. Influence of conservative retinoblastoma treatment in the occurrence of metastasis

While reducing the enucleation rate, the transition from one therapeutic era to the next also brought great concerns regarding a potential negative impact on the “state of metastatic grace”, an empiric window for globe preserving therapies, where the risk for metastasis is not higher than the risk encountered following enucleation with adjuvant therapy in case of histopathologic high risk factors. Fear of inappropriate management of secondary enucleated eyes due to downstaging after chemotherapy on the one hand (Zhao et al., 2011), and of the possible consequences of lower systemic chemotherapy exposure in patients treated with intra-arterial chemotherapy alone on the other hand (Yousef et al., 2016), is nourished by the lack of well-designed studies aimed at capturing metastatic events and related deaths.

Among the few studies which have addressed this crucial issue, two compared the incidence of metastatic disease and orbital recurrence in advanced retinoblastoma group D/E treated with

either systemic or intra-arterial chemotherapy versus primary enucleation and concluded to the absence of variation by treatment modality (Berry et al., 2017b; Yannuzzi et al., 2015). In another paper comparing patients primarily treated by external beam radiotherapy versus first line chemotherapy (Chantada et al., 2014), the authors failed to detect a change in the probability of extraocular relapse, despite the prediction of a putative positive effect of chemoreduction in preventing systemic metastasis in children with microscopically disseminated disease. The controversy regarding the rate of metastases following first line intra-arterial versus intravenous chemotherapy for advanced disease clashes with biases linked to the fact that these two administration routes influence the ratio of CNS versus non-CNS dissemination (Brennan et al., 2015). Neoadjuvant systemic chemotherapy may offer a less favorable coverage in patients with high risk for CNS involvement when compared to the unmatched chemotherapeutic optic nerve concentration achieved by intra-arterial drug delivery (Taich et al., 2016), while intravenous chemotherapy may be more efficient against distant minimally disseminated disease. Although the end result in terms of survival remains unknown to date, patient survival following the introduction of first line intra-arterial chemotherapy appears, however, unimpaired compared to previous treatment regimens, with no increase in the metastasis rate, ranging between 2 and 2.5% (Funes et al., 2018) and death rate from metastatic disease around 1% (Abramson et al., 2017b). Noteworthy, in all cases, the cause of metastases resulting in death was parental refusal of enucleation of a single remaining eye (Abramson et al., 2017b).

3.8. Second primary neoplasms

Retinoblastoma patients with a germline *RB1* mutation are at lifetime risk to develop various types of second primary (Abramson et al., 1979; Eng et al., 1993; Wong et al., 1997) and even third, fourth and fifth malignancies (Abramson et al., 2001; Marees et al., 2010) (see 3.8.1). This

risk is further increased after external beam irradiation and, to a lesser extent, chemotherapy (see 3.8.3.2). In middle and high-income countries where the majority of patients survive retinoblastoma, second primary tumors represent the leading cause of death in patients with hereditary retinoblastoma (Kleinerman et al., 2005; Marees et al., 2009; Moll et al., 1997; Moll et al., 2001). Despite various follow-up strategies for retinoblastoma survivors (Baker et al., 2016), specific screening protocols are still missing (see 3.8.4).

3.8.1. Incidence and mortality of second primary neoplasms

According to long-term retinoblastoma survivor cohort studies from around the world (Marees et al., 2010), the risk of second primary neoplasms is much higher for hereditary than for non-hereditary retinoblastoma survivors, for whom the standardized incidence ratio is comparable to that of the general population (Table 8). This risk also increases significantly with a longer follow-up, in particular for epithelial cancers such as lung, bladder or breast carcinomas. Thus, according to a Dutch study, the 40-year absolute excess risk of second primary neoplasms in hereditary retinoblastoma survivors was 261 excess cases per 10,000 survivors per year with a cumulative incidence of 28% (95% confidence interval=CI (21.0-35.0%)) (Marees et al., 2008).

The cumulative mortality from second primary malignancies 50 years after retinoblastoma diagnosis was 25.5% (95% CI (21-30%)) in hereditary versus 1% (95% CI (0.2-1.8%)) in non-hereditary retinoblastoma survivors (Marees et al., 2009; Yu et al., 2009).

3.8.2. Characteristics of second primary neoplasms

Second primary tumors can develop in diverse anatomic locations, including the skull, bones, soft tissues, nasal cavity, skin, orbit, brain, breast, bladder and lung. In a review of 676 secondary primary malignancies in 602 retinoblastoma survivors, the most prevalent neoplasms were sarcoma (68%), carcinoma (14%), melanoma (8%), leukemia and lymphoma (4%) (Woo

and Harbour, 2010). The latency to develop a second tumor depends on the secondary primary tumor type and previous treatment. The median age at diagnosis was 13 years for sarcoma, 27 years for melanoma and 29 years for carcinomas (Woo and Harbour, 2010). In a cohort of German pediatric retinoblastoma survivors, the most prominent second primary neoplasms were sarcoma and leukemia with a standardized incidence ratio of 148 (95%CI (39.81-378.87)) and 41 (95%CI (11.13-105.95)) respectively (Temming et al., 2015). In this study, a different latency in the diagnosis was observed for solid versus non-solid second primary neoplasms, diagnosed in children older or younger than 5 years of age, respectively (Temming et al., 2015). However, in this cohort, the increased risk of leukemia could be due to the adjunct of systemic alkylating agents in the German protocol (Kunkele et al., 2015).

The diagnosis of a second primary neoplasm needs to be confirmed by cytopathologic analysis of a tumor biopsy. Small round blue cell tumors such as lymphoma, rhabdomyosarcoma or nephroblastoma, however, can be hard to distinguish from metastatic retinoblastoma. In such cases, the presence or absence of previously identified somatic *RBI* mutations and/or retinoblastoma-specific somatic copy number changes can help differentiate second cancers from metastatic disease (Racher et al., 2016).

3.8.3. Factors influencing the risk of second primary neoplasms

3.8.3.1. Genetic predisposition

Retinoblastoma survivors carrying a germline mutation, treated with enucleation or focal treatment with no additional radiation or chemotherapy, are clearly at increased risk to develop second primary tumors (Abramson et al., 1979; Eng et al., 1993; Wong et al., 1997).

In a study investigating variations in second cancer risk by family history of retinoblastoma among long-term survivors, the authors concluded that carriers of an inherited germline mutation

are at slightly higher risk compared with those with a *de novo* germline mutation (Kleinerman et al., 2012), which may be partially attributed to mosaicism or reduced penetrance (Moll et al., 2012). Preliminary research also suggested an increased second primary malignancy risk in a group with a nonsense *RB1* mutation (hazard ratio or HR=3.53 (95% CI (1.82-6.84)) compared to bearers of a low penetrance *RB1* mutation (HR=0.19 (95% CI (0.05-0.81)) (Dommering et al., 2012). However, this observation was recently challenged, at least in irradiated hereditary patients, suggesting no protective effect in low penetrant *RB1* mutation carriers (Chaussade et al., 2018).

3.8.3.2. Influence of therapy

3.8.3.2.1. Radiotherapy

External beam irradiation increases the risk of developing second primary malignancies in patients with hereditary retinoblastoma threefold (Kleinerman et al., 2005; Marees et al., 2008; Temming et al., 2017), especially bone cancers and soft tissue sarcomas in a dose-dependent manner (Fletcher et al., 2004; Wong et al., 1997), or if given before the age of one year (Abramson and Frank, 1998; Moll et al., 2001). More recent radiotherapeutic modalities with proton radiotherapy and/or stereotactic conformal radiotherapy are expected to induce fewer second primary neoplasms in hereditary retinoblastoma survivors compared to conventional radiotherapy (Munier et al., 2008; Pica et al., 2011; Sethi et al., 2014).

3.8.3.2.2. Chemotherapy

There is still little long-term information about the contribution of chemotherapy alone on the risk of second primary malignancies in hereditary retinoblastoma patients. The cumulative incidence ratio in hereditary retinoblastoma treated with chemotherapy alone (3.0, CI 0.3-10.8) appears, however, to be comparable to patients treated with enucleation or focal treatment alone

and 3 times lower compared to patients receiving external beam irradiation (Temming et al., 2017). Some have reported a potential acute risk to develop at an early time point acute myelogenous leukemia after systemic chemotherapy (Chantada et al., 2014; Gombos et al., 2007; Temming et al., 2015), while this was not confirmed by others for chemoreduction with vincristine, etoposide and carboplatin (Turaka et al., 2012). However etoposide-related secondary leukemias with 11q23 involvement have been reported in the literature even in patients who received what are considered safe cumulative dosages for other pediatric tumors (Chantada et al., 2014; Gombos et al., 2007).

Targeted chemotherapy (periocular, intra-arterial and intravitreal) does not seem to increase the rate of secondary primary malignancies (Ishida et al., 2016; Suzuki et al., 2011), although follow-up time is too short to draw definite conclusions. Recently, a review of 214 patients with hereditary retinoblastoma treated by intra-arterial chemotherapy over a 10-year period revealed a comparable rate of second primary neoplasm to previously published ones (Habib et al., 2018).

3.8.4. Long-term follow-up in heritable retinoblastoma survivors

While some institutions routinely perform annual MRI of the head or total body MRI screening (Friedman et al., 2014), others perform complementary exams only when indicated. In general, retinoblastoma survivors undergo as many, but not more, cancer screening tests compared to the general population (Sheen et al., 2008). Regular skin examination for melanoma is important, especially for patients with impaired vision. Overall, the most important aspect of screening starts by educating the patient, family and (primary) doctors on the increased risk and on common signs of second primary tumors (Dimaras et al., 2015).

4. Retinoblastoma genesis

Like any cancer, the biological and clinical behavior of retinoblastoma is dictated by the unique features of the cancer cells and their interactions with the host. In turn, the cancer cell features are largely determined by the intrinsic gene expression program of the retinoblastoma cell-of-origin in combination with changes to the cell-of-origin signaling circuitry that are induced by oncogenic mutations. However, in contrast to most cancers, the genetic changes that initiate retinoblastoma are well defined, and the mechanisms through which the retinal cell-of-origin transforms into a cancer are coming into focus. This chapter describes how our understanding of retinoblastoma genesis has come about, including the identification of the *RB1* gene, genomic alterations beyond *RB1* mutations, the origin of retinoblastoma from cone photoreceptor precursors, and collaboration of *RB1* mutations with the underlying cone precursor circuitry.

4.1. Mutational inactivation of the *RB1* gene initiates retinoblastoma tumorigenesis

Based on a statistical study of the distribution of the number of tumors and age at diagnosis in 48 patients with retinoblastoma, Alfred Knudson hypothesized that retinoblastoma is caused by two mutational events (Knudson, 1971). It was not part of Knudson's original hypothesis that these alterations target two alleles of an autosomal gene locus. However, analysis of genotype changes at genetic polymorphisms in tumors, specifically the pattern of loss of constitutional heterozygosity (LOH), indicated that two mutational events target a gene on chromosome 13q (Cavenee et al., 1983).

In 1986, fifteen years after Knudson's initial paper, *RB1* was identified as the gene that is the target of two-step mutational inactivation in retinoblastoma (Friend et al., 1986; Fung et al., 1987; Lee et al., 1987). Fulfilling the predictions of Knudson's hypothesis, heritable and non-

heritable retinoblastoma are distinct with respect to the timing of the two mutational events that are required to alter both *RB1* alleles (see 5.1).

In more recent years, comprehensive genomic analysis has confirmed that, as far as genetic alterations are concerned, bi-allelic inactivation of *RB1* may be sufficient for retinoblastoma genesis (Grobner et al., 2018; Zhang et al., 2012). Indeed, somatic chromosomal copy number alterations are absent in most retinoblastomas from children who are diagnosed at an early age (Herzog et al., 2001), and exome sequencing analyses revealed that some early tumors lack gene mutations other than *RB1* as well as chromosomal copy number alterations (Kooi et al., 2016a). However, the question of whether this implies that all retinoblastomas initially form without further genomic alterations remains open.

4.2. Most retinoblastomas show more than two hits

Although early tumors may develop with biallelic *RB1* loss as the sole genomic alteration, cytogenetic meta-analyses of several hundred retinoblastomas have shown recurrent chromosome 1q, 2p, and 6p gains and 16q losses (Corson and Gallie, 2007; Kooi et al., 2016b). Genomic gains on the short arm of chromosome 2 appear to target the *MYCN* locus. The targets of genomic gains at 1q and 6p are not well-defined, but initial candidates included *MDM4*, *KIF14* on 1q and *DEK* and *E2F3* on 6p (Corson and Gallie, 2007). A meta-analysis identified other genes in the peak gain regions with significant gene-dosage effects, including *ZBTB41*, *CRB1* and *NEK7* on 1q and *SOX4* on 6p (Kooi et al., 2016b). Numerous genes have been proposed to underlie the frequent 16q loss such as *CDH11*, encoding a cadherin possibly involved in cell-cell attachments and *RB1-like 2* (*RBL2*) encoding the pRB-related p130 protein, which have decreased expression in tumors with 16q loss (Kooi et al., 2016b). These chromosome 16q changes appear to be associated with vitreous seeding, yet a causal link has not

been established (Gratias et al., 2007). Exome analyses also revealed recurrent focal deletion or mutational inactivation of *BCOR*, a gene on the X chromosome that encodes a non-canonical polycomb repressor complex 1 component in about 10% of retinoblastomas and a recurrent mutation of *CREBBP* at even lower frequency (Kooi et al., 2016a; Zhang et al., 2012).

At present, it is unclear whether the recurrent genomic changes (1q, 2p, and 6p gains, 16q losses, and *BCOR* and *CREBBP* inactivation) are required for the initial development of the retinoblastomas in which they are found, or if they are selected after the initial appearance of a tumor in order to mediate more rapid growth. Motivated by the observation that genomic gains and LOH are often present in addition to bi-allelic *RBI* gene inactivation, a multistep model of development of retinoblastoma was suggested (Corson and Gallie, 2007; Gallie et al., 1999). Further support for such a model was drawn from the observation that samples from tumor areas that were classified as retinomas based on histomorphologic findings, showed bi-allelic *RBI* inactivation and relatively few chromosomal copy number changes, whereas adjacent areas with histomorphologic features of retinoblastoma had more chromosomal gains and losses (Dimaras et al., 2008b; Sampieri et al., 2009). Likewise, the larger tumors typical of later diagnosis have more chromosomal alterations as well as decreased differentiation-related gene expression, suggesting that retinoblastomas progress via sequential acquisition of chromosomal and gene expression changes (Cobrinik, 2015; Kooi et al., 2015). However, subsequent analyses indicated that chromosomal change did not appear in a particular order (Kooi et al., 2016b). Thus, the multistep evolution of retinoblastoma may differ from the prototypical adenoma-carcinoma sequence in colon cancer in which chromosomal alterations appear in a specific sequence (Vogelstein et al., 1988).

A better understanding of retinoblastoma progression may come from studies of retinoblastoma tumor DNA obtained via paracentesis of aqueous humor. Recently, it was shown that DNA in the aqueous humor resembles that of enucleated tumors and can change over time (Berry et al., 2017c). In one case, a recurrent chromosomal change (6p+) was not detected in the earliest sample but appeared in subsequent aqueous humor taps, suggesting that this change was selected during tumor evolution, albeit under chemotherapy selection. Further analyses of copy number alterations in multiple aqueous humor samples beginning at the time of diagnosis, may reveal the order(s) in which they occur during conservative treatment (see 8.4).

4.3. RB1 mutations initiate tumorigenesis in the retinoblastoma cell-of-origin – the cone photoreceptor precursor.

The malignant behavior of retinoblastoma depends on the effects of *RB1* loss and other genomic alterations superimposed on the cell signaling circuitry of the retinoblastoma cell-of-origin. Here, we recount efforts to identify the cell-of-origin, the evidence favoring or disfavoring a cone photoreceptor origin, and the potential contributions of the cell-of-origin circuitry for retinoblastoma initiation and clinical behavior.

4.3.1. First hints pointing towards a photoreceptor cell-of-origin

The identity of the retinoblastoma cell-of-origin has long been debated, in part because early inferences were based on analyses of tumor cells that were far removed from tumor initiation events. Briefly, the tumors were initially referred to as “*glioma of the retina*” based on use of imperfect stains that suggested a glial composition. In the 1890s, Flexner and Wintersteiner independently recognized that a subset of the tumors had symmetrically arranged structures – later termed “Flexner-Wintersteiner rosettes” – that appeared to be composed of photoreceptor-like cells and were suggestive of a rod or cone origin (Flexner, 1891; Wintersteiner, 1897).

However, most tumors lacked Flexner-Wintersteiner rosettes while others had “Homer Wright rosettes” that are also seen in diverse non-retinal tumors (Albert, 1987), which argued against a photoreceptor origin. Eventually, the term “*retinoblastoma*” was adopted based on the perceived similarity of the tumor cells to embryonic retinal progenitor cells (Albert, 1987; Verhoeff and Jackson, 1926), yet this hardly settled the cell-of-origin question.

Decades later, a growing body of evidence emerged suggesting a predominant cone photoreceptor phenotype in retinoblastoma. Specifically, researchers detected an intact cone but not rod phototransduction cascade as well as high-level expression of RNAs and proteins mediating cone phototransduction in both differentiated and undifferentiated tumors (Bogenmann et al., 1988; Hurwitz et al., 1990; Rodrigues et al., 1992). However, the cone phenotype remained arguable, with evidence that retinoblastoma cells might simultaneously express non-cone markers and recognition that some retinoblastoma cell phenotypes could be adopted after transformation of an unrelated retinal cell type. Further evidence of a cone origin came from the finding that retinoblastoma foci are distributed over the retinal surface in a pattern similar to that of L/M cones (Curcio et al., 1990; Munier et al., 1994), although the cone-like distribution could also have other explanations (King et al., 2015).

4.3.2. Efforts to identify the retinoblastoma origin using genetically engineered mouse models

The lack of progress in identifying the cell-of-origin based on characteristics of retinoblastoma tumors prompted the adoption of mouse modeling approaches that held potential to reveal inaugural steps in retinoblastoma initiation. However, this approach also ran into difficulties, as no retinal tumors formed in *Rb1*^{+/-} mice (Clarke et al., 1992; Jacks et al., 1992; Lee et al., 1992). Furthermore, mice with retina-targeted mutation of *Rb1* and constitutive

germline mutation of the *Rb1*-related *p107* or *p130* formed retinal tumors with a predominant amacrine or horizontal cell phenotype, rather than the cone photoreceptor phenotypes that seemed to predominate in human retinoblastoma (Cobrinik, 2013; Macpherson, 2008). Perhaps, the different human *vs.* mouse phenotypes could have been reconciled if tumors in both species derived from retinal progenitor cells yet took on different retinal cell-type-specific features. However, the data showed that retinal progenitor cells in *Rb1*^{-/-} as well as in *Rb1*^{-/-};*p107*^{-/-} retinæ were largely unaffected, whereas diverse post-mitotic retinal neurons re-entered the cell cycle, proliferated, and underwent apoptosis to varying extents (Chen et al., 2004). Concordantly, mice with retina-specific inactivation of *Rb1* in a *p130*^{-/-};*p107*^{+/-} background appeared to initiate tumorigenesis in horizontal cell interneurons only after terminal differentiation and synaptogenesis had begun (Ajioka and Dyer, 2008). Thus, models with combined *Rb1* family mutations revealed that retinal tumors can develop from post-mitotic interneurons that aberrantly re-enter the cell cycle, rather than from retinal progenitor cells that fail to exit the cell cycle. Moreover, the resulting mouse tumors retained features of their interneuron cell-of-origin, at odds with the notion that retinal tumors dramatically alter their phenotype during tumorigenesis.

4.3.3. Circumstantial and direct evidence for a cone precursor cell-of-origin

The disparate retinal phenotypes that had been observed in retinoblastoma tumors prompted a re-examination of the retinal cell-type-specific protein expression (Xu et al., 2009). The studies focused on retinal cell-type-specific transcription factors and distinguished neoplastic retinoblastoma cells, which are deficient in the *Rb1*-encoded pRB protein, from pRB⁺ non-neoplastic cell infiltrates. Analyses of 40 retinoblastomas revealed that the vast majority of pRB⁻

tumor cells expressed the cone-specific cell lineage factors TR β 2 and RXR γ , the cone+rod+bipolar cell-specific CRX transcription factor, and the cone-specific phototransduction proteins cone arrestin and L/M-opsin. Rare pRB⁻ cells co-expressed S-opsin and L/M-opsin, consistent with the occasional S- and L/M-opsin co-expression in developing L/M cones (Cornish et al., 2004). All of these proteins were expressed at levels comparable to the cone precursors in developing retina. In contrast, only rare and non-neoplastic pRB⁺ cells in rare tumors expressed markers of rods, retinal ganglion cells, amacrine or horizontal cells, or retinal progenitor cells at levels comparable to the developing retina (including NRL, rhodopsin, Brn3b, syntaxin, Prox1, Chx10, and Pax6). Moreover, combined immunostaining and *in situ* hybridization revealed that rare cells that lacked pRB and cone marker expression retained two *RB1* alleles, implying that only cone-marker⁺ cells had biallelic *RB1* loss (Xu et al., 2009). These findings indicated that pRB-deficient and *RB1*-mutant retinoblastoma cells express numerous proteins that are characteristic of L/M-cones but not markers specific to other retinal cell types.

In keeping with the predominant cone protein expression profile of the pRB⁻ cells, the Muller cell and astrocyte markers Nestin and GFAP were consistently detected only in non-neoplastic pRB⁺ cells (Xu et al., 2009). The Nestin⁺ and GFAP⁺ cells also expressed PAX2, indicative of derivation from non-neoplastic retinal astrocytes, and expressed the astrocyte and neural stem cell determinant SOX2 (Xu et al., 2010), consistent with the detection of other neural stem cell markers in a subset of cells in retinoblastoma tumors (Seigel et al., 2007). Importantly, SOX2 was only detected in cells expressing pRB protein and/or retaining *RB1* alleles, establishing the lack of this stem cell marker in neoplastic retinoblastoma cells (Xu et al., 2010).

The neoplastic retinoblastoma cells' cone protein expression profile provided one of several lines of circumstantial support for a cone precursor cell-of-origin. For example, a cone origin was also consistent with the high-level pRB expression observed in maturing human cone precursors (Lee et al., 2006) but not in maturing mouse cone precursors (Spencer et al., 2005; Xu et al., 2009). Moreover, maturing human but not mouse cone precursors prominently expressed the proto-oncoproteins MDM2 and MYCN (Xu et al., 2009). As most tumors require the inactivation of the p53 pathway as well as the RB pathway (Sherr and McCormick, 2002), the cone precursors' high-level MDM2 expression provided a way to suppress p53 function without mutation of the *TP53* gene or *MDM2* amplification. However, the cone precursor's expression of these oncoproteins provided only circumstantial evidence of a cone origin, as their expression could have been acquired during the development of retinoblastoma from an alternative cell type.

More recent studies provided direct evidence for the cone precursor cell-of-origin. Specifically, pRB knockdown elicited cell cycle entry and proliferation of human cone precursors but not other retinal cell types (Xu et al., 2014). The cone precursor response to pRB knockdown was evident in dissociated retinal cultures, in prospectively isolated cone precursors, and in non-dissociated retinal tissue. Moreover, cell cycle entry depended on the cone cell lineage factors TR β 2 and RXR γ and on the cone-precursor-expressed MYCN, whereas the survival of the aberrantly proliferating cells depended on the endogenous MDM2. Notably, co-depletion of 130 along with pRB enhanced proliferation, consistent with evidence that the *RBL2/p130* gene on 16q is often lost in retinoblastoma. Finally, pRB-depleted and pRB/p130-depleted cone precursors formed retinoblastoma-like tumors in orthotopic xenografts, documenting the cone precursors' capacity to serve as a retinoblastoma cell-of-origin.

4.3.4. *Reconciling the cone precursor cell-of-origin with apparently contradictory observations*

Despite the direct evidence of the cone precursors ability to form retinoblastoma-like tumors (Xu et al., 2014), the cone origin theory - if true – must be reconciled with all relevant biologic and clinical observations. In this regard, two observations that initially seemed to contradict the cone cell-of-origin theory are addressed here.

First, the earliest lesion detected in retinoblastoma patients via OCT appear to be centered in the inner retina, either in the inner nuclear layer or in the outer plexiform layer, rather than in the outer aspect of the outer nuclear layer where cones reside (Berry et al., 2016a; Rootman et al., 2013). This location suggested that the tumors either derive from a non-cone cell in the inner nuclear layer (which might subsequently adopt a cone phenotype) or derive from cones that are displaced towards the inner nuclear layer, perhaps through a rare but normal phenomenon (Semo et al., 2007) or in response to pRB loss. Recently, the movement of pRB-depleted cone precursors from the outer edge of the outer nuclear layer to the outer plexiform layer were detected via live imaging of intact cultured retinæ, in support of the cone precursor cell-of-origin concept (Singh et al., 2018a).

A second concern was that retinoblastoma tumor cells express RNAs that encode proteins that are characteristic of diverse retinal cell types (McEvoy et al., 2011). The expression of non-cone-related RNAs appeared to conflict with evidence that retinoblastoma cells mainly express cone-related proteins (Xu et al., 2009) and suggested that the retinoblastoma cell-of-origin might have adopted a hybrid gene expression program, with expression of cone as well as non-cone genes, after the transformation of non-cone cell type. However, the data is also consistent with the expression of a hybrid program after transformation of cone precursors in combination with

preferential translation of cone-related RNAs. More studies are needed to resolve the discrepancy in the expression of non-cone RNAs with the predominant cone-related protein profile in retinoblastoma cells.

Finally, if cone precursors represent the cell-of-origin, pRB depletion may be expected to induce not only cone precursor proliferation but also the different tumorigenesis stages that have been inferred from clinical observations. With regard to this hypothesis, non-proliferative *RB1*^{-/-} retinoma lesions were found to be present at the base of some, and perhaps most, retinoblastoma tumors (Dimaras et al., 2008a), suggesting that small retinoma-like lesions might be present but imperceptible at birth and later reactivate to form retinoblastoma tumors. Thus, if cone precursors do represent the cell-of-origin, pRB depletion would be expected to induce not only cone precursor proliferation but also a proliferative arrest with retinoma-like features, followed by retinoblastoma-like tumors. Indeed, pRB depletion was found to enable cone precursor proliferation, a retinoma-like arrest, and the emergence of retinoblastoma-like masses in long-term human retina cultures (Singh et al., 2018b). The retinoblastoma-like masses emerged with timing that was similar to that of retinoblastomas in babies, consistent with them representing an authentic retinoblastomagenesis process (Fig 7).

4.4. Collaboration between *RB1* inactivation and the cell-of-origin circuitry.

The evidence of the cone precursor origin of retinoblastoma inevitably provokes the question of why the tumors derive from this cell type. More specifically, why is *RB1* loss sufficient to initiate retinoblastoma with exceptionally high penetrance in cones but not in all other retinal cell types? While the answers are still emerging, it appears likely that cone precursor circuitry is oncogenic in-and-of-itself, and collaborates with *RB1* loss to mediate cell cycle re-entry. As

mentioned above, the cone precursors have high-levels MDM2 and MYCN, which may subvert p53-mediated tumor suppression and promote proliferation, respectively (Fig 7). Moreover, MDM2 and MYCN may be part of a wider cone precursor proliferation-related program (Singh et al., 2016) that is ultimately linked to the transcriptional programming of the cells. For example, Xu et al. found that the human *MDM2* gene is regulated by the cone-specific transcription factor RXR γ (Fig 7) and suggested that the nearly perfect RXR γ consensus binding site in the human *MDM2* promoter underlies high-level MDM2 expression (Xu et al., 2009). More recent studies suggest that the wider cone precursor proliferation-related program is limited to a single cone maturation stage (Singh et al., 2018b). The manifestation of this cone precursor state in humans, but not other species, appear to confer the uniquely human susceptibility to retinoblastoma as well as the unique characteristics of retinoblastoma tumors.

5. Retinoblastoma genetics and genetic counseling

In this part are described the different variants of heritable (50%) and non-heritable (50%) (MacCarthy et al., 2009) presentations of retinoblastoma, as well as their implications for the patient's family members, based on family history, laterality at presentation, and current genetic tests.

5.1. Genetic presentations of retinoblastoma

Retinoblastoma can be classified into four distinct genetic presentations according to the timing of the first *RBI* mutation:

1. *familial*, or *inherited heritable* form, in which the retinoblastoma patient has inherited a predisposition to retinoblastoma from an affected parent;

2. *isolated (or sporadic) heritable* form, in which retinoblastoma is typically bilateral with no family history resulting from a *de novo* pre-zygotic germline mutation;
3. *mosaic* form, in which retinoblastoma is usually unilateral, caused by a post-zygotic mutation, and heritable only if the germline is also involved;
4. *non-heritable* forms, in which retinoblastoma is always unilateral, unifocal and sporadic, resulting from a somatic mutation.

The heritable form of retinoblastoma – either familial or isolated – is caused by germline genetic alterations that disable the *RBI* gene functions, also referred to as oncogenic variants or alleles. Individuals with familial or isolated heritable retinoblastoma are heterozygous for an oncogenic *RBI* allele (the “first mutation”), throughout their body (Fig 8A). Each individual retinoblastoma focus that develops in such individuals is initiated by an additional genetic alteration targeting the other *RBI* allele (the “second mutation”) during retinal development. Thus, expressivity varies according to the number of independent second mutations that give rise to tumor foci. In addition, expressivity can also vary according to the nature of the first mutation, which determines the complete or incomplete penetrance of the inherited or *de novo* predisposition, as described below.

5.1.1. *Inherited heritable retinoblastoma (familial retinoblastoma)*

Familial occurrence of retinoblastoma is a form of heritable retinoblastoma in which predisposition to this tumor is transmitted as an autosomal dominant trait. In the familial setting, retinoblastoma screening may be initiated pre- or perinatally and therefore usually associated with a presymptomatic diagnosis (see 3.5). In high-income countries, the fraction of familial

cases among heritable retinoblastoma is estimated around 25%, but is rapidly rising due to improved life prognosis.

5.1.1.1. Familial retinoblastoma with complete penetrance

In most pedigrees with familial retinoblastoma, all family members who are heterozygous for an oncogenic *RB1* allele develop at least one retinoblastoma focus (complete penetrance, Fig 8Ba). Typically, patients develop several tumors and, consequently, bilateral retinoblastoma is prominent in most families. As a rule, a high probability of tumor development is caused by genetic alterations that result in complete or almost complete loss of pRB protein function, which are most often nonsense alterations, frameshift-indels, and alterations at canonical splice signals that result in frameshift exon skipping. In these pedigrees, the mean number of tumors per patient is around 6 (Abramson and Gombos, 1996; Munier et al., 1994).

5.1.1.2. Familial retinoblastoma with incomplete penetrance

In some pedigrees, heritable retinoblastoma shows incomplete penetrance with some family members free of any tumor, despite being heterozygous carriers of an oncogenic *RB1* allele (Fig 8 Bb). Incomplete penetrance is a consequence of lower probability of tumor development and those patients typically develop fewer retinoblastoma foci compared to those with complete penetrance, associated with genetic alterations that retain some of the pRB protein functions or affect regulation of transcription of this gene (reviewed in (Lohmann, 2004 #1178). Consequently, patients in families with incomplete penetrance also tend to show only unilateral retinoblastoma. Most of the difference in penetrance of heritable retinoblastoma can be explained by differences in the functional consequences of oncogenic *RB1* alterations. The most prominent examples of variant *RB1* alleles associated with a low penetrance phenotype are certain missense

variants (e.g. LRG_517p1:p.R661W), in-frame indels, alterations at remote splice signals, specific variants in exon 1, and alterations of transcription-factor binding sites in the *RB1* promoter.

Families with lower probability of tumor development provide a good opportunity to identify factors in *trans* to the *RB1* locus that may affect intra-familial variation of phenotypic expression. The minor allele of rs2279744 (NM_001145337.2:c.-291T>G), a single-nucleotide polymorphism in the *MDM2* promoter known to be linked to enhanced MDM2 expression, has been reported to act as a modifier of phenotypic expression in heritable retinoblastoma (Castera et al., 2010). Parent-of-origin effects have been identified as another source of intra-familial variation of phenotypic expression (Klutzn et al., 2002), found later to be related to human *RB1* gene imprinting (Eloy et al., 2016; Kanber et al., 2009).

Identification of the mechanisms underlying phenotypic expression, namely low-penetrance *RB1* alleles, *trans*-acting modifiers, and epigenetic factors, is not an end in itself. It can be anticipated that some of the mechanisms mediating milder phenotypic expression will be used to reduce the number of tumor foci, in family members at risk.

5.1.2. Isolated heritable retinoblastoma

In this presentation, retinoblastoma occurs in a child of unaffected parents. Isolated heritable retinoblastoma results from a *de novo* pre-zygotic mutation in the gametes of one of the healthy parents. According to the nature of the first mutation, expressivity – defined as the number of primary tumor foci – may be attenuated or not.

5.1.2.1. Isolated bilateral retinoblastoma (full expressivity)

Most patients with newly diagnosed bilateral retinoblastoma have no relatives with retinoblastoma and are referred to as having isolated or sporadic bilateral disease (Fig 8 Bd).

Most patients (95%) with isolated bilateral disease are heterozygous for an oncogenic *RBI* allele (Lohmann and Gallie, 2004) and, consequently, also have heritable disease, but occasionally (5%) may be mosaic (see 5.1.3). Typically, oncogenic alleles identified are the result of *de novo* mutations (Fig 8 Ab). They are usually indistinguishable from *RBI* alleles underlying familial retinoblastoma with complete penetrance, and result in complete or almost complete loss of pRB protein functions (see 5.1.1.1).

Due to the non-familial occurrence, patients with isolated bilateral retinoblastoma will not have been formally screened for retinoblastoma during infancy, and the age at diagnosis reflects the mean time to elicit symptoms, which is usually around 9 months (Goddard et al., 1999). In this population, the mean number of tumors per patient is 6, which is not different from that observed in familial retinoblastoma patients with complete penetrance (personal observation).

5.1.2.2. Isolated retinoblastoma with genomic 13q14 deletion (reduced expressivity)

Some patients with germline deletions include *RBI* as well as neighboring genes on chromosome 13q. Those have a lower probability of tumor development and thus show an excess of unilateral presentations (40%) (Munier et al., 1989) compared to patients carrying other types of loss of function alleles. The mean age at diagnosis in this population is around 17 months (Baud et al., 1999; Munier et al., 1989), which is intermediate between isolated heritable and non-heritable *RBI*-related retinoblastoma. It appears that the risk to develop retinoblastoma in carriers of deletions larger than 1Mb containing the *MED4* gene is reduced compared to non-chromosomal mutation carriers (Mitter et al., 2011), with a mean number of tumors per patient of 2.5 (FLM personal observation) versus 6.3 (Munier et al., 1994) respectively. The observed 61% reduction of tumor number in 13q- patients actually closely corresponds to the 63% predicted tumorigenic event due to loss-of-heterozygosity (Zhu et al., 1992), those turning lethal by

nullisomy of housekeeping genes in retinoblastoma 13q- precursor cells. Individuals with large interstitial 13q deletions show a developmental syndrome resulting from the haplo-insufficient loss of the affected 13q genes and display a recognizable midfacial dysmorphic phenotype (Motegi et al., 1983; Munier et al., 1989; Bojinova et al., 2001).

5.1.3. *Mosaic retinoblastoma*

In familial retinoblastoma with complete penetrance it is not unusual to find that the first affected family member (i.e. the founder on the phenotype level), presents with unilateral retinoblastoma while affected family members in subsequent generations show bilateral disease (Fig 8 Bc). Genetic analysis has shown that some of these founders are somatic mosaics for an oncogenic *RBI* allele (Fig 8 Bc) (Sippel et al., 1998). In these individuals, the first mutation that led to the oncogenic *RBI* allele was not received via the germline but occurred somatically after conception, giving rise to a mutant sector of variable extent according to the timing of the event. A mutational event that occurs during early stages of intrauterine development may result in a mutant sector in the individual. The number of tumor foci in those patients is expected to be lower because the probability of initiation of retinoblastoma development in an individual with somatic mosaicism is reduced inasmuch as the mutant sector contributes only partially to the developing retina.

Mosaicism for an oncogenic *RBI* allele is estimated to account for 5% of patients with isolated bilateral disease (Fig 8 Ac) and 20% of the cases with isolated unilateral retinoblastoma (Lohmann D personal communication). If the proportion of the mutant allele present in DNA from peripheral leucocytes is low, this may result in false negative findings in genetic testing. The absence of detectable mosaicism, however, does not rule out somatic mosaicism, since the cells carrying the mutant *RBI* allele might not be detected in the sampled cells (blood, buccal

etc), but might be present in other tissues such as the retina or germ cells. Thus, there is no test that excludes risk of retinoblastoma in the unaffected eye or, if the mutant sector includes germline cells, in offspring of the patient. However, risk estimates are lower in unilateral retinoblastoma patients who test negative for constitutional *RBI* mutation compared to the pretest state of knowledge.

5.1.4. Non-heritable retinoblastoma

5.1.4.1. Isolated unilateral retinoblastoma with somatic biallelic *RBI* mutation

In about half of newly diagnosed retinoblastoma patients only one eye is affected and family history is negative (isolated unilateral disease, Fig 8 Be). In nearly 90% of these patients, genetic analysis of DNA from retinoblastoma shows genetic or epigenetic alterations of both *RBI* alleles that, upon targeted genetic testing, are not detected in DNA from blood (Schuler et al., 2005). This shows that both alterations are the result of mutation events that occurred in somatic cells but does not rule out somatic mosaicism instigated by the first mutation. In the remaining 10% of isolated unilateral retinoblastoma a *de novo* germline mutation can be identified.

In non-heritable retinoblastoma, there is only one tumor in the affected eye (*i.e.*, there is a unilateral unifocal phenotype). The mean age at diagnosis in this population is 24 months (Goddard et al., 1999), although an earlier age at presentation is not necessarily suggestive of a germline or mosaic *RBI* mutation carrier (Schuler et al., 2005).

5.1.4.2. Retinoblastoma without alterations of the *RBI* gene

In less than 3% of patients with isolated unilateral retinoblastoma, analysis of DNA from the tumor shows no alteration at the *RBI* locus. However, nearly half of these tumors (1.4%) show high amplification levels of a genomic region on the short arm of chromosome 2 that includes

the *MYCN* gene (Rushlow et al., 2013). Although some retinoblastomas with *RBI* gene inactivation also show increased *MYCN* copy numbers, tumors without *RBI* mutations have gained a far higher number of *MYCN* copies. The distinct genetic makeup of *MYCN*-amplified tumors is reflected by a clinical phenotype marked by very early age at diagnosis of less than 6 months and histopathologic features reminiscent of neuroblastoma. Thus, it might be appropriate to regard this genetic variant as a distinct entity, possibly linked to a distinct genesis. However, a recent report suggests that tumors from *MYCN* transduced retinal cells not only appear earlier than those with *RB*-depletion, coinciding with earlier age at diagnosis in *MYCN*-induced retinoblastoma patients, but also derive from a cone precursor cell of origin (Singh et al., 2019).

5.2. Genetic counseling and testing

Initial counseling and the testing approach for each individual patient depends on the clinical presentation, including familial versus isolated background and bilateral versus unilateral appearance. The three presentations do not necessarily reflect the genetic types of retinoblastoma described in 5.1, as sporadic bilateral or unilateral retinoblastoma can result from several genetic mechanisms.

5.2.1. Familial retinoblastoma

For pre-test genetic counseling and recurrence risk prediction in typical situations of familial bilateral retinoblastoma it is reasonable to assume a probability of transmission of 50% (autosomal dominant inheritance) and a probability of tumor development of 100% (complete penetrance in heterozygous individuals). Consequently, the estimated pretest recurrence risk of heritable retinoblastoma in offspring of a family member with bilateral retinoblastoma is 50% at birth. Although indirect genetic testing by linkage analysis of marker alleles linked to the *RBI*

gene may be available, most laboratories prefer direct genetic testing by determining the presence or absence of the oncogenic heritable predisposition in a given family.

A wide spectrum of genetic alterations can result in oncogenic *RB1* alleles (allelic heterogeneity) (Valverde et al., 2005). Various methods are required to cover the relevant genomic regions and the different types of potential oncogenic alterations (small mutations, copy number changes, rearrangements).

In almost all pedigrees with apparent complete penetrance and bilateral retinoblastoma, current routine methods will identify a pathogenic allele (>95%), provided that the mutation screening analysis is performed on a sample from an affected family member who has inherited the retinoblastoma predisposition.

In rare pedigrees with complete penetrance retinoblastoma, no oncogenic *RB1* allele is identified, due to inactivating alterations not covered by current routine technology (e.g. in frame copy number, neutral inversions), but destined to be diagnosed in the near future with diagnostic sensitivity improvement, especially with the implementation of genomic sequencing technology for routine testing.

In pedigrees where incomplete penetrance is obvious, diagnostic sensitivity of genetic testing is somewhat lower compared to families with complete penetrance. This is because there are fewer constraints regarding the spectrum of alterations that result in only partial inactivation of pRB protein functions compared to alterations that result in full inactivation. As current routine methods used for mutation screening do not cover all regions that may be affected by oncogenic alterations with incomplete penetrance (e.g. deep intronic variants, regulatory variants outside of known transcription factor binding sites), some oncogenic alterations are missed.

Another point that contributes to reduced diagnostic sensitivity of genetic testing in familial retinoblastoma with incomplete penetrance is the uncertainty regarding the pathogenetic role of variants of unknown significance, even if cosegregating with the heritable predisposition.

Although there is no valid report of familial retinoblastoma not genetically linked to the *RBI* locus, locus heterogeneity cannot be formally ruled out. What has been reported, however, are observations of fortuitous co-occurrence of retinoblastoma in relatives (Dryja et al., 1993; Munier et al., 1993).

5.2.2. *Isolated bilateral retinoblastoma*

The empirical recurrence risk for siblings of a sporadic bilaterally-affected patient is 2% (Draper et al., 1992) and this figure reflects both the low frequency of germinal mosaicism in one of the parents and the lower than 50% probability of transmission of a mutant allele in these situations. It is rare to find an unaffected parent heterozygous for a variant that confers high probability to tumor formation. Consequently, recurrence risk in more remote family members (e.g. cousins) is low. Pretest recurrence risk in offspring of a patient with isolated bilateral retinoblastoma is expected to be 50% at the time of birth, assuming a 50% probability of transmission of 100% probability of tumor development. However, as some patients with isolated bilateral retinoblastoma have somatic mutational mosaicism, the overall probability of transmission is less than 50%.

In principle, the strategies of genetic testing are analogous to those in familial retinoblastoma. With current routine methods, an oncogenic variant is identified in DNA from blood of almost 95% of patients with isolated bilateral retinoblastoma, which is lower compared to familial retinoblastoma, due to somatic mosaics with low proportions of mutation-carrying leukocytes. Although this limitation can be overcome by deep sequencing technology, at least for some types

of alterations such as single nucleotide variants, robust strategies for technical validation of low-level findings remains to be established. In most patients, the signal ratio of wild type and variant alleles in DNA from blood suggests a heterozygous genotype. However, checking a second source of constitutional DNA (e.g. DNA from buccal swabs or finger nails) is a worthwhile adjunct analysis as it may detect possible somatic mosaicism. If informed consent is granted, parents should be tested for the presence of the variant allele. Most loss of function-type alleles appear to have occurred *de novo*, and are preferentially found on the allele of paternal origin (Hagstrom and Dryja, 1999; Zhu et al., 1989).

In some patients, the signal ratio of wild type and variant alleles in DNA from blood is imbalanced. Such a finding may be due to somatic mosaicism, but further genetic testing is required to validate this interpretation. However, if somatic mosaicism is confirmed this excludes an increased risk in siblings and relatives of the patient's parents. Therefore, the additional efforts required to confirm somatic mosaicism in a patient with isolated bilateral retinoblastoma are often justified.

Another option to overcome the limitations of diagnostic sensitivity due to somatic mosaicism is to include analysis of tumoral DNA. Analytically, the most reliable source of tumor DNA is a fresh frozen sample obtained at the time of enucleation. If the tumor mutations are not identified, constitutionally heterozygous intragenic polymorphic *RBI* markers can be used looking for a loss of heterozygosity (LOH) in the tumor, as seen in 63-70% of the cases (Cavenee et al., 1983; Zhu et al., 1992). Whenever LOH is present, the recurrence risk is linked to the transmission of the allele retained in the tumor. If tumor DNA markers are not informative, or in the absence of enucleation, the known paternal preferential origin of *de novo* germline *RBI* mutants, seen in

90% of the cases, can be used to modulate the recurrence risk, which is maximized if the paternal or grand-paternal allele is passed to a sib of an offspring respectively (Tran et al., 2012).

If the oncogenic *RBI* variant responsible for the heritable predisposition is known in the index patient, genetic counseling in relatives is as straightforward as in familial retinoblastoma (see above), with two notable exceptions: i) If testing of DNA from blood of parents did not show the variant allele identified in the child, this will reduce but not exclude increased risk in siblings. The remaining risk is due to germline mosaicism, i.e. the presence of cells with the mutant allele in germline cells of a parent. Unfortunately, empirical data required to quantify the risk due to germline mosaicism are still missing. ii) The second caveat relates to the origin of chromosomal aberrations such as genomic deletions at 13q14 (see 5.1.2.2). If a chromosomal alteration identified in a child is not present in either parent, this does not exclude the presence of aberrations causing unbalanced ones. Chromosome analysis in parents can detect such aberrations in some families.

5.2.3. Isolated unilateral retinoblastoma

Pretest genetic counseling has to take into account that various genetic mechanisms may result in isolated unilateral retinoblastoma. The empirical recurrence risk figures provided by Draper, which integrate all alternative causes, are still valid for risk estimation in siblings and offspring of patients with isolated unilateral retinoblastoma (Draper et al., 1992). The low empirical recurrence risk for siblings (1% at time of birth) reflects that heterozygosity with incomplete penetrance or germline mosaicism for an oncogenic allele in a parent is rare. Moreover, although the probability of transmission of an oncogenic allele from a rare heterozygous parent is 50%, recurrence risk is further reduced because of the lower probability of tumor development caused by alleles associated with incomplete penetrance. Risk for the

patient's first child is higher (6% at time of birth) and, in most cases, is due to somatic mosaicism in the unilaterally affected parent. Because of somatic mosaicism, probability of transmission is reduced (overall <50%). However, the oncogenic alleles transmitted by such parents are typically associated with high probability of tumor development. This explains why tumors in children of patients with isolated unilateral retinoblastoma typically show bilateral disease.

The results of genetic testing can help to identify which of the alternative genetic mechanisms has resulted in isolated unilateral disease in a given patient. In fewer than 15% of patients, constitutional heterozygosity for an oncogenic *RB1* allele is detected. Genetic counseling of these families must take into account estimates of disease penetrance as indicated by the functional type of mutation. The most frequent genetic mechanism (in over 80% of isolated unilateral patients) is somatic inactivation of both alleles of the *RB1* gene. This diagnosis excludes an increased recurrence risk in siblings. If testing shows no evidence of somatic mosaicism then pretest risk in offspring is reduced (possibly less than 1%), with the option to exclude an increased risk after predictive genetic testing for heritable oncogenic alterations that had been identified in the parent's tumor. Of note, although some somatic alterations that cause *RB1* inactivation in retinoblastoma are not heritable (e.g. chromothripsis (McEvoy et al., 2014)) and epigenetic silencing (Greger et al., 1994), it is still possible that the propensity to acquire such alterations is influenced by heritable genetic variation.

In rare cases, genetic analysis of the patient's retinoblastoma shows no alteration at the *RB1* locus but high-level amplification of the *MYCN* gene. Current data and models strongly suggest that these tumors are distinct entities without recurrence risk for the unaffected eye of the patient, nor for his or her relatives. However, to date, the genetic diagnosis of *MYCN*-retinoblastoma has

not been validated medically. Moreover, prior to utilizing the results for predictive testing, it would be reassuring to have results from longitudinal observations of patients and their families. Recognition of this specific entity is likely to be possible even in conservatively-treated eyes based on liquid biopsy, by paracentesis of aqueous humor as shown for copy number variations (Berry et al., 2018b).

6. Clinical growth and seeding patterns

The retinoblast undergoing malignant transformation initiates a primary intraretinal tumor, which has not only the capacity to metastasize but also, unlike other cancers, to generate secondary tumors by seeding. Clinical features of retinoblastoma retinal growth and seeding patterns are reviewed below.

6.1. Retinoblastoma growth patterns

Primary tumors generated by retinal oncogenesis are characterized clinically by 6 distinct growth patterns, namely endophytic, exophytic, cavitory, diffuse infiltrating, anterior diffuse and spontaneously regressed retinoblastoma. Note that on top of this phenotypic retinal repertoire, retinoblastoma can also result from extra-retinal oncogenesis leading to ectopic growth in the pineal gland and supra/parasellar region (trilateral retinoblastoma) (see 3.6).

6.1.1. Classic growth patterns of primary retinoblastoma: endophytic, exophytic, mixed growth

Incipient retinoblastoma can be traced by OCT to the inner nuclear layer or in the outer plexiform layer (see 4.3.4). By definition, tumor progression towards the inner or outer retina is associated with endophytic or exophytic retinoblastoma respectively (Fig 9 A, B), but the link with the OCT variants remains unknown.

Endophytic retinoblastoma presents as a whitish mass rapidly interrupting the retinal vasculature and protruding into the vitreous cavity secondary to alterations of the vitreo-retinal interface by two distinct mechanisms (Fig 9 A):

- (i) At the tumor apex, by tumoral infiltration of the internal limiting membrane and hyaloid, leading to vitreous seeding (see 6.2.1).
- (ii) At the tumor base, by tractional detachment of the hyaloid, creating a new ocular compartment for retrohyaloid seeding (see 6.2.2).

Finally, note that in case of endophytic growth, the retina contiguous to the tumor base can also undergo a localized circumferential tractional detachment with well-demarcated boundaries.

Exophytic retinoblastoma presents as a mass beneath the retina, sparing the retinal surface, which retains unaltered retinal vasculature (Fig 9 B). This growth variant impacts the retinal pigment epithelium-photoreceptor complex by causing an exudative retinal detachment, under which subretinal seeding can occur. The detachment, initially intermittent, position-dependent, and leaving poorly defined demarcation lines on the retina, can become total and bullous with further tumor growth, inducing complete spontaneous vitrectomy when the retina enters in contact with the lens (Fig 9 D).

Mixed endophytic and exophytic growth is a feature of more advanced retinoblastoma (Fig 9 C), often associated histopathologically with choroidal infiltration across the RPE and Bruch's membrane. This uveal invasion tends to occur more often with exophytic than endophytic retinoblastoma (Nawaiseh et al., 2015; Palazzi et al., 1990; Shields et al., 1993a). Endophytic and exophytic retinoblastoma can also coexist in the same eye.

6.1.2. Rare variants of retinoblastoma

In addition to the common endophytic and exophytic growth patterns, 3 other variants of retinoblastoma can be encountered, accounting for about 5% of the cases at presentation, namely cavitory (2.7-4%) (see 6.1.2.1), diffuse infiltrating (1-2%) (see 6.1.2.2), and diffuse anterior retinoblastoma (<1%) (see 6.1.2.3). All these subtypes are characterized by an atypical presentation, often causing a delayed diagnosis or even life-threatening invasive investigations or therapeutic procedures. Awareness of characteristic features described below help the early recognition, crucial to provide appropriate management.

6.1.2.1. Cavitory retinoblastoma

Cavitory retinoblastoma is a rare low-grade endophytic growth variant with intratumoral pseudo-cysts visible as grey lucent cavities at the tumor surface on ophthalmoscopy, appearing hypofluorescent on fluorescein angiography, and as empty spaces on ultrasonography (Palamar et al., 2008) (Fig 9 E, F). Cavitory retinoblastoma typically displays a mean number of 2-3 cavitations of an average diameter of 3 mm (Chaudhry et al., 2018; Rojanaporn et al., 2012). Diagnosis is made at a mean age of about 15 months and predominates in bilaterally affected patients in approximately 2/3 of the cases (Chaudhry et al., 2018; Rojanaporn et al., 2012). These tumors may co-exist with non-cavitory retinoblastoma tumor foci in the same or fellow eye. Secondary intracavitory seeding has been observed in 10% (n=2/20) of the cases followed in Lausanne (unpublished data) (see 6.2). Noteworthy, cavitory retinoblastoma may develop new cavities during treatment, and secondary cavities may become visible in an initially non-cavitory tumor after an average of two courses of chemotherapy (Chaudhry et al., 2018). On the other

hand, cavities can collapse under treatment as seen in 44% of the eyes after a mean follow-up of 18 months (Chaudhry et al., 2018).

In contrast to non-cavitary retinoblastoma where systemic or intra-arterial chemotherapy induces a mean tumor reduction of approximately 30-35% in basal diameter and 50-56% in thickness (Shields et al., 2011; Shields et al., 1996), cavitary tumors typically show shrinking only by half these values as a mean (Rojanaporn et al., 2012), and sometimes not at all (Fig 9 E,F). In addition, relapse of cavitary retinoblastoma appears to be exceptional, occurring in none of 18 eyes (0%) after a mean follow-up of 40 months in a series from London (Chaudhry et al., 2018), and in only one of 26 eyes (4%) in the series from Philadelphia after a mean follow-up of 4 years (Rojanaporn et al., 2012), again very different from the relapse rate of 20-28% characterizing non-cavitary retinoblastoma (Gombos et al., 2002; Shields et al., 2011; Shields et al., 2004b).

In general, despite apparent resistance, cavitary retinoblastoma does not require aggressive or prolonged treatment. However, considering a recurrence risk estimate of about 4%, similar to that of retinoma, life-long follow-up is advised (Abouzeid et al., 2012; Balmer et al., 1991; Singh et al., 2000) (see 6.3).

6.1.2.2. *Diffuse infiltrating retinoblastoma*

Diffuse infiltrating retinoblastoma is another rare variant found in 1-2% of the cases, characterized by a planar horizontal growth rather than the vertical growth seen in endophytic and exophytic retinoblastoma (Fig 9 G). Typically, the tumor infiltrates the retina which appears thickened on B-scan, UBM or MRT, without visible mass or calcifications. Histopathologic reports have shown diffuse tumoral invasion of the ganglion cell layers (Fernandez et al., 2017), appearing as a hyper-reflective homogenous thickening on OCT (Stathopoulos et al., 2018c) (Fig

9 H, I). This OCT signature was shared in all four diffuse infiltrating tumors investigated so far in Lausanne (Stathopoulos et al., 2018c; FLM unpublished data). Interestingly, a type 0 regression with complete restoration of the retinal microanatomy was observed on OCT after intra-arterial and intravenous chemotherapy (Stathopoulos et al., 2018c) (Fig 9 H', I').

Compared to classic retinoblastoma, the diagnosis of diffuse infiltrating retinoblastoma is usually made in older children (mean age 5.7 years) following atypical presenting symptoms such as vision loss, ocular redness or pain, in the context of misleading signs most frequently masquerading inflammation or pointing to other diagnoses, including neovascular glaucoma and cataract (Traine et al., 2016). In addition to frequent diagnostic delay, slow tumor growth could explain the older age at presentation (Bhatnagar and Vine, 1991; Traine et al., 2016). Most cases (>90%) are unilateral and sporadic. Diffuse infiltrating retinoblastoma and typical retinoblastoma have, however, been observed in the same patient (FLM personal observation) or within family members carrying the same mutation (Kao, 2000; Schedler et al., 2016), raising the question of a possibly distinct genesis in this atypical disease subtype. As retinal specific micro-environmental features are known to contribute to retinoblastoma tumorigenesis (Xu et al., 2010), a specific tropism of infiltrating retinoblastoma tumor cells for the ganglion cells favoring this atypical growth over the inner plexiform layer can be hypothesized.

Until recently, all cases have been managed with primary enucleation (Bhatnagar and Vine, 1991; Fernandez et al., 2017; Foster and Mukai, 1996; Shields et al., 2008). Last year, for the first time, one successful case of eye salvage with vision preservation was reported after focal treatment and intra-arterial chemotherapy at an updated 38-month follow-up (Stathopoulos et al., 2018c). Optical coherence tomography provides instrumental information to monitor the tumor front line following treatment in such cases.

6.1.2.3. *Diffuse anterior retinoblastoma*

The term diffuse anterior retinoblastoma was first proposed in 1998 to describe an exceedingly rare variant of the disease (Grossniklaus et al., 1998), totalizing only 13 published cases up to now (Jijelava and Grossniklaus, 2013; Kelly et al., 2016; Munier et al., 2017a; Shields et al., 2016c). Diagnosis occurs usually later than the other forms (mean age 6.4 years). Half of the cases present with a cellular infiltration of the anterior chamber without retinal or vitreous involvement, while in the other half, a small retinal lesion is found at the ora serrata, typically with concomitant vitreous seeding (Jijelava and Grossniklaus, 2013; Kelly et al., 2016; Munier et al., 2017a; Semb et al.; Shields et al., 2016c).

Diffuse anterior retinoblastoma has been considered a variant of diffuse infiltrating retinoblastoma, originating either from a very small focus close to the ora serrata (Jijelava and Grossniklaus, 2013) or directly from the anterior segment (Shields et al., 2016c). In our opinion, however, this subtype of retinoblastoma constitutes a unique, separate entity, originating from two distinct sources found at the extreme fundus periphery (Fig 10), explaining the two different clinico-pathologic manifestations observed in this subtype of retinoblastoma, namely without or with minimal peripheral retinal involvement. Hence, in the case of diffuse anterior retinoblastoma without retinal tumor, we think that the tumor arises from ectopic retina found anteriorly to the ora serrata, corresponding either to retinal islands embedded in the pars plana (Eisner, 1973) or to cristae retinae (Fig 10). As both structures lack the internal limiting membrane (Daicker, 1972), a tumor originating from this ectopic retina is expected to seed immediately into the posterior chamber without growing a mass (Fig 10 A-C). Conversely, in the case of anterior diffuse retinoblastoma with a minimal peripheral retinal involvement, we believe that the tumor originates from the retinal rim found under the vitreous base (Fig 10), where L/M

cones are known to out-number rods (Ahnelt, 1998; Williams, 1991). Since the retinal rim under the vitreous base is devoid of hyaloid and has a thin fenestrated inner limiting membrane (Le Goff and Bishop, 2008), a tumor arising from this location will initially grow as an endophytic mass which can rapidly invade both posterior chamber and vitreous cavity (Le Goff and Bishop, 2008) (Fig 10 D-F).

6.2. *Intra-ocular seeding*

Seeding of tumor cells is a characteristic feature of retinoblastoma. It can, however, be observed in other primary intra-ocular tumoral conditions such as melanocytoma of the optic nerve (Shields et al., 2006c), ciliary body medulloepithelioma (Kaliki et al., 2013a), retinal astrocytoma (Cohen et al., 2008), and uveal melanoma (Metz et al., 2016).

Seeding of the primary tumor engenders no less than 12 recognizable phenotypes, comprising all possible combinations of the 3 known classes of seeds, i.e. dust, spheres and clouds, across the 4 intraocular seeding compartments, i.e. the vitreous, aqueous, as well as the subretinal and retrohyaloid spaces (Francis et al., 2015a; Munier, 2014).

6.2.1. *Vitreous seeding*

Vitreous seeding occurs following the disruption of the inner limiting membrane of the retina and hyaloid either by spontaneous or by iatrogenic mechanisms. Interestingly, transmission electron microscopy measurements showed that the thickness of the internal limiting membrane is characterized by huge topographic variations ranging from 20 nm at the foveola to 2500 nm in the macular region and decreasing progressively with greater eccentricity down to 50 nm at the vitreous base (Foos, 1972), where the inner limiting membrane is perforated (Le Goff and Bishop, 2008). The inner limiting membrane opposes therefore less resistance to seeding in the

periphery, accounting for the preferential anterior location of class 3 seeds as recently reported (Francis et al., 2016). Schematic representation and peripheral clinical limits of intravitreal seeding in endophytic retinoblastoma growth pattern is shown in Figure 11 A-C.

Spontaneous vitreous seeding can be present at diagnosis (primary seeding) as a pathognomonic feature of endophytic retinoblastoma, or complicate the disease course at tumor relapse (secondary seeding) independently of the initial growth pattern (Munier, 2014). In the first scenario, by far the most frequent, the passage of tumor cells into the vitreous is part of an active infiltration at tumor apex, discharging individual cells (class 1 seeds). The ability of these cells to survive and grow under the hypoxic vitreous conditions will depend on metabolic reprogramming and/or resistance to anoikis. In other words, only a fraction of these cells will acquire the clonal capability to form spheres (class 2 seeds). According to their adhesion-independent or dependent properties, they will give rise to free floating spheres or hemispheres anchored to the inner face of the hyaloid. In the second scenario, the passage into the vitreous follows a passive mechanism by mechanical or necrotic disruption of the tumor apex, leading to the formation of a cloud (class 3 seeds). The translocation of the tumor content by gravity into the vitreous body is sometimes endowed of gravitational motility, thus masking or unmasking the retina depending on the head position (Fig 12.A-C). Here again the translocated tumor mass is mostly composed of dead cells (Amram et al., 2017) and only a minority will survive to produce spheres (class 2 seeds).

Iatrogenic (secondary) vitreous seeding is a well-known complication of inappropriate energy transfer by photocoagulation or diode-mediated hyperthermia (Gombos et al., 2006), followed by dispersion of tumor cells (class 1 seeds) into the vitreous. It can also occur in the context of intravenous (Parness-Yossifon et al., 2009) or intra-arterial chemotherapy (De Francesco et al.,

2014), with the sudden formation of class 3 seeds consecutive to internal limiting membrane disruption following rapid tumor regression. Finally, indentation during ophthalmoscopic examination of endophytic tumors at diagnosis, or during positioning of a plaque, may also be at the origin of iatrogenic tumor dispersion.

6.2.2 *Retrohyaloid seeding*

Retrohyaloid seeding is underdiagnosed and often confounded with vitreous seeding. The retrohyaloid space is generated secondary to rapid endophytic growth and formation of a tractional hyaloid detachment, or to the inability of the contracted vitreous to re-expand and compensate the collapsed tumor volume after treatment (Fig 12 D-F). The extent of the retrohyaloid volume can be evaluated by ultrasonography, occupying up to half of the vitreous volume. The recognition of a hyaloid detachment is crucial in order to avoid grade 4 or 5 melphalan-induced retinopathy linked to inadvertent retrohyaloid injection of intravitreal chemotherapy (see 7.3.5.1.2.2).

The retrohyaloid seeds can float freely (Fig 12 I-K), or attach to the inner limiting membrane of the retina and/or the outer hyaloid face. Class 3 retrohyaloid seeding typically appears as a circular position-dependent cloud, masking the optic nerve and fovea only in dorsal decubitus (Munier, 2014) (Fig 12 D-H). Schematic representation and peripheral clinical limits of retrohyaloid seeding in endophytic retinoblastoma growth pattern is shown in Figure 11 D-F.

6.2.3 *Subretinal seeding*

Subretinal seeding is the hallmark of exophytic retinoblastoma. Two distinct presentations of class 2 subretinal seeding can be distinguished, i.e. spheres usually anchored to the detached outer retina, or flat placoid lesions with a tendency to confluence in a shallow subretinal space (Fig 11 G-I). The area of initial retinal detachment remains at risk for persistent or recurrent

subretinal seeding following first line treatment. It is important to keep in mind the full extent of the detached retinal territory, sometimes difficult to identify due to the on/off phenomenon seen in exudative retinal detachment, in order to distinguish new peripheral tumors from recurrent subretinal seeds.

By gravity, class 2 seeds tend to accumulate inferiorly just posterior to the ora serrata (Fig 11 G-I). In the case of bullous retinal detachment, the seeds attach to the outer retina and can project anterior to the ora serrata. When the retina is folded over the pars plana, UBM is the only way to differentiate these subretinal ante-oral tumors from an invasion of the posterior chamber.

6.2.4. Aqueous seeding

Aqueous seeding refers not only to the presence of retinoblastoma tumor cells in the anterior chamber on biomicroscopy (Fig 13), but also to its invisible posterior chamber counterpart. Chronologically, invasion of the posterior chamber typically occurs first, leaving the anterior chamber in a transient cryptic seeding state before converting to an overt involvement. In case of infiltration of the iris root by (choroido)-ciliary or supraciliary mass, however, the passage of retinoblastoma cells to the anterior chamber can bypass the posterior chamber (see 6.2.4.3).

Ultrasonographic biomicroscopy is instrumental to document anterior segment tumor involvement, enabling in particular to determine whether the aqueous seeding is isolated or associated with an anterior uveal infiltration of the ciliary body and/or iris (Fig 14).

6.2.4.1. Prevalence of anterior chamber seeding

Anterior chamber seeding at diagnosis is rarely seen in high-income countries, being present in approximately 1% of the cases (Haik et al., 1987), except in two rare subtypes of retinoblastoma, namely diffuse infiltrating (Shields et al., 2008) and anterior diffuse retinoblastoma (Jijelava and Grossniklaus, 2013), where it is found in 65% and 100% of the

cases, respectively. The presence of anterior chamber seeding automatically grades disease as group E according to the International Intraocular Retinoblastoma Classification (IIRC), with primary enucleation recommended (Murphree, 2005).

Secondary anterior chamber seeding appears at a mean interval of 28 months (range 3–99) after diagnosis (Munier et al., 2018), and was found in 6% of secondary enucleated eyes with advanced retinoblastoma treated with chemoreduction (Shields et al., 2009), and 23.5% (Kiratli et al., 2017) to 50% (Pavlidou et al., 2015) of those treated with intra-arterial chemotherapy.

Until recently (see 7.2.4), secondary anterior chamber seeding has always been managed by enucleation (De Francesco et al., 2014; Parness-Yossifon et al., 2009).

6.2.4.2. *Prevalence of anterior uvea invasion*

Anterior uvea (iris and ciliary body) invasion, isolated or combined with aqueous seeding is reported in 2-7% of primary enucleated eyes in developed countries (Brennan et al., 2015; Kaliki et al., 2013b; Uusitalo et al., 2001), versus 8-12% of primary enucleated eyes in developing countries (Kaliki et al., 2015b). In a recent series directly comparing primary and secondary enucleations, the authors described a significantly higher incidence of anterior segment involvement (17% vs 0%, $p < 0.05$) following secondary enucleation (Fabian et al., 2017c). Others reported similar findings with a higher frequency of ciliary body invasion in secondary enucleated eyes having received neoadjuvant chemotherapy (13%) compared to primary enucleation (7%), and hypothesized that this increase in anterior histopathologic features may reflect a differential targeting of chemotherapy against ciliary body compared to posterior uvea, due its poorer vascular supply (2 long posterior vs 20 short posterior ciliary arteries) (Brennan et al., 2015).

6.2.4.3. Invasion mechanisms of the aqueous humor

Aqueous humor invasion can occur via five different pathways, as documented on clinical, ultrasonographic (UBM) and histopathologic observations (Fig 15):

- (i) *Trans-hyaloid invasion* from tumor cells originating from vitreous seeds anchored to the anterior hyaloid, or from peripheral parietal tumors growing over the pars plana (Fig 15 E-H).
- (ii) *Trans-ciliary invasion* via growth of a ciliary/choroido-ciliary tumor directly into the posterior or anterior chamber and /or by infiltration of the iris root (Fig 15 A, B).
- (iii) *Iatrogenic invasion* by tumor cells freely invading the posterior chamber from the posterior segment after mechanical disruption of the anterior hyaloid, e.g. post pars plana vitrectomy, endocapsular lensectomy or posterior capsulorhexis.
- (iv) *Epiciliary invasion* by tumor cells originating from an oral or ectopic retinal source, as seen in diffuse anterior retinoblastoma (see 6.1.2.3) (Fig 10).
- (v) *Supra-ciliary invasion* by anterior chamber angle infiltration of a supra-ciliary mass (Fig 15 C, D).

In a consecutive series of 33 eyes with anterior segment involvement followed in Lausanne, the most frequently observed invasion mechanism was transhyaloid, seen in 67% (n=22/33), followed by trans-ciliary (12%, n=4/33) and iatrogenic routes (12%, n=4/33). The epiciliary and the supraciliary invasion were observed only in 6% (n=2/33) and 3% (n=1/33), respectively (unpublished data).

Recognition of the invasion mechanism underlying aqueous humor invasion is determinant for the adequate treatment strategy, as the seeding source must be sterilized concomitantly to the aqueous seeding, which became treatable only recently with intracameral chemotherapy (*see*

7.2.4) (Fig 16). Whereas iris invasion can be controlled by intra-arterial melphalan (Munier et al., 2018), ciliary body involvement needs to be treated by brachytherapy alone (Chhablani et al., 2010) if isolated, or by combining brachytherapy and intracameral chemotherapy if associated with aqueous seeding (Munier et al., 2018).

6.3. Retinoma/retinocytoma and phthisis bulbi

Retinoma, also called retinocytoma, retinoblastoma group 0, and spontaneously arrested or regressed retinoblastoma, is used to designate a benign phenotypic counterpart of retinoblastoma. The pathologic nature of these lesions was described in 1983 as benign photoreceptor proliferations with numerous fleurettes and absence of necrosis or mitotic activity (Margo et al., 1983). More recently, review of histopathologic sections of enucleated eyes showed areas with features of retinoma in 15 to 20% of them, suggesting evolution from retinoma to retinoblastoma (Dimaras et al., 2008b; Eagle, 2009).

6.3.1. Prevalence and relationship with retinoblastoma

The exact frequency of retinoma in the population is not known. Half of the reported cases are diagnosed by routine examination of first degree relatives of retinoblastoma probands, especially in familial cases (Abouzeid et al., 2012; Gallie et al., 1982). In a series of 171 sporadic retinoblastomas, retinoma was diagnosed in only 2 asymptomatic progenitors (FLM personal observation). The reported proportion of retinoma among the population with retinoblastoma varies from 1.8% (17/920) to 3.2 % (16/505) (Abouzeid et al., 2012; Singh et al., 2000). Retinoma can also be suspected retrospectively in late-onset diagnosed retinoblastoma in older children or adults (Eagle et al., 1989; Mataftsi et al., 2012), or in tumors with no or minimal initial response to chemoreduction and absence of growth after discontinuation of all treatments (Chung et al., 2010; Shields et al., 2002).

Like retinoblastoma, retinoma can be present in one or both eyes. The lesion can also co-exist with retinoblastoma in the same or fellow eye. In retinoma germline carriers, the underlying disease-predisposing *RBI* mutations are indistinguishable from those found in retinoblastoma patients (Abouzeid et al., 2009), indirectly confirming that *RBI* double hits are necessary but not sufficient to cause retinoblastoma (Dimaras et al., 2008b).

6.3.2. Clinical features and risk of malignant transformation

The majority of the lesions correspond to spontaneously arrested and/or regressed proliferations, appearing typically as a translucent retinal mass with or without calcifications and/or retinal pigment epithelial alterations (Gallie et al., 1982). In a report on 86 retinoma foci in 49 eyes, 40% of the retinoma foci showed regression patterns types I and IV, and 60% types II and III, including 5 tumors with pseudo-cyst, among which 2 displayed benign cavitory enlargement over time (Abouzeid et al., 2012).

Phenotypic variability also includes spontaneously regressed vitreous seeds (Abouzeid et al., 2012; Hadjistilianou et al., 2006; Lueder et al., 1995; Shah et al., 2011), regressed subretinal seeds, clinical signs of past retinal detachment and histopathologic evidence of full thickness choroidal and prelaminar optic nerve head invasion (Abouzeid et al., 2012) (Fig 17 A, B). Such findings suggest the existence of a more aggressive retinoma subtype prior regression than typical ones. Finally, phthisis bulbi has been reported in 6.6 to 8.3% in the two largest series of patients with spontaneously regressed retinoblastoma (Abouzeid et al., 2012; Gallie et al., 1982).

The mechanisms underlying regression/growth arrest of retinoma remain to be determined. Considering the occasional multifocal and/or bilateral disease, however, such a coordinated growth stop of millions of cells across different ocular compartments is more likely to be caused

by a still unknown humoral or circulating factor, than explained by a cell-intrinsic mechanism or vascular collapse.

As retinoma can undergo secondary malignant transformation with an estimated lifelong risk of about 5%, regular control of the lesions are needed (Abouzeid et al., 2012; Singh et al., 2000) (Fig 17 C-E).

7. Conservative management of intra-ocular retinoblastoma

With the emergence of new therapies over the last 20 years, the management of retinoblastoma has also become more complex. The first rule remains the defense of life, which presupposes a permanent arbitration between pursuit of conservative therapy and secondary enucleation. This being said, one of the most important keys to success is continuous adaptation of the therapeutic strategy to the different eye compartments involved (see 3.2). The right decision-making implies a solid knowledge of the strengths and limits of the different modalities that have come to enrich the therapeutic armamentarium of retinoblastoma, but also the awareness of their potential complications. Efforts to implement eye-conservative strategies in less developed countries should consider local socio-economic and cultural factors as well as availability of resources for treatment and patient support. In many of these settings, compliance with follow-up visits and timely acceptance of enucleation when conservative therapies have failed may not be comparable to higher income countries, causing an increased risk for disease progression and death. In addition, even mild chemotherapy regimens may be associated to increased risk of toxic death in these settings (Chantada et al., 2013).

This part deals first with an overview of the pharmacokinetics of the most commonly used chemotherapeutic drugs in retinoblastoma treatment according to the different routes of delivery (see 7.1), illustrating why the development of targeted routes of chemotherapy administration

directly into the eye was essential to achieve control of tumor seeding. We then review the different available therapeutic modalities and their contribution in reducing the enucleation rate and eradicating the need for external beam radiotherapy (see 7.2). Furthermore, major disease- and treatment-related intraocular complications encountered during conservative retinoblastoma management are highlighted to bring into perspective one of the biggest remaining challenges of disease management, namely vision preservation (see 7.3). Finally, this section ends with a review on the quality of life (see 7.4).

7.1. Pharmacokinetics.

To achieve the intended goal, chemotherapeutic drugs must become available in a sufficient concentration of their active form at the intended tumor site. In parallel, for retinoblastoma patients, it is also important to take into account the amount of drug in the bloodstream to counterbalance efficacy with systemic toxicity. The vitreous-to-plasma and the retina-to-plasma drug exposure ratios are important concepts to define the most selective route for ocular drug delivery and to avoid exposure of normal tissues to antineoplastic agents.

Despite the use of chemotherapy in the conservative treatment of retinoblastoma for more than 20 years, knowledge about the intraocular bioavailability of the different drugs remains incomplete. Moreover, information, mainly based for ethical reasons on animal studies, is often characterized by discrepancies with the clinical observations secondary not only to differences in the anatomy between animal and human eyes, but probably also between non-tumor bearing and tumor-bearing-eyes, whose integrity of the blood-ocular barrier is compromised.

The following overviews the main pharmacokinetic studies with emphasis on the different possible administration routes of the chemotherapeutic drugs used against retinoblastoma. A schematic representation of previously published data of chemotherapy ocular exposure in

different animal models after several local and systemic routes of drug delivery is presented in Figure 18.

7.1.1. Intravenous drug delivery

To penetrate the different ocular compartments after intravenous delivery, the drugs have first to make it through the first-pass metabolism and, once in the choroidal and retinal vessel, to overcome the blood-ocular barrier. The latter consists posteriorly of the tight junctions of the retinal pigment epithelium and the retinal endothelial cells and, anteriorly, of the inner non-pigmented ciliary epithelium, the posterior iris epithelium, and the endothelium of the iris and ciliary body vessels. High doses of chemotherapy are consequently required intravenously to attain pharmacologically active concentrations in the ocular tissues, carrying simultaneously the risk of severe systemic adverse events (Del Amo et al., 2017; Edelhauser et al., 2010).

Systemic chemotherapy as conservative retinoblastoma treatment (typically with carboplatin, etoposide, and vincristine) was introduced in the 1990s, without preclinical studies on intraocular pharmacokinetics and remains the most frequently used treatment worldwide even though only a small fraction of the administered drugs is thought to make its way through the first-pass metabolism and the blood-ocular barrier.

Specifically, studies in tumor-bearing juvenile rats showed that intravenous carboplatin, a cell-phase nonspecific alkylating agent, achieved an intravitreal exposure of 60% of the systemic area under the curve (AUC) (Laurie et al., 2005), whereas in a non-human primate model, the maximum achieved concentration in the vitreous (C_{\max}) was only 1% of the plasma C_{\max} but, surprisingly, the aqueous humor C_{\max} was 20% of the systemic level (Mendelsohn et al., 1998). Interestingly, the concentration of carboplatin in the vitreous in enucleated eyes from retinoblastoma patients was 13-fold higher than that attained after the same dosage in non-human

primates (Abramson et al., 1999a), but similar to the levels obtained by others in rabbits subjected to cryotherapy (Wilson et al., 1996).

Pharmacokinetic studies with intravenous etoposide, a topoisomerase II inhibitor, performed in rats and non-human primate models have shown a very low to undetectable vitreous exposure compared to the systemic exposure (Laurie et al., 2005; Mendelsohn et al., 1998).

Intravenous vincristine, a cell-mitosis inhibitor from the vinca alkaloid family, exhibited good retinal and vitreous exposure in rats, almost equal to plasma exposure, but the assays performed were not able to distinguish between a degraded or protein-bound form of the molecule (Laurie et al., 2005).

The low selectivity of the intravenous route for chemotherapy delivery into the vitreous was also reported for topotecan, a topoisomerase type I inhibitor, in non-tumor-bearing rats and rabbits with a vitreous-to-plasma exposure ratio of less than 0.4 (Carcaboso et al., 2007; Laurie et al., 2005). Nonetheless, intravenous topotecan is still used in the clinics for retinoblastoma treatment (Brennan et al., 2017).

From the above, it appears that other administration routes had to be explored to improve tumor control, especially in advanced disease with seeding into the different ocular compartments.

7.1.2. Intra-arterial drug delivery

The technique of the super-selective catheterization of the ophthalmic artery was developed to allow the injection of high drug concentration directly into the artery that irrigates the ocular tissues, thus avoiding the first-pass elimination and reducing at the same time systemic exposure to the cytotoxic drug. The hypothesis of an increased bioavailability in the ocular tissues with greater selectivity compared to systemic exposure was confirmed four years after the initial

publication on 9 cases treated this way (Abramson et al., 2008), in pharmacokinetic animal studies using non-tumor bearing pigs (Schaiquevich et al., 2012a; Schaiquevich et al., 2012b).

The most frequently injected drug in the ophthalmic artery is melphalan, a nitrogen mustard agent found to be the most efficient drug among the 12 tested against retinoblastoma cells *in vitro* (Inomata and Kaneko, 1987). Its cytotoxic activity is mediated by alkylating nucleic acids and proteins, which alters DNA function and ultimately leads to cell death. Rapid spontaneous hydrolysis of the reconstituted drug from its commercial vial implies infusion within 1.5 hours in order to avoid the loss of the active moiety.

Another frequently injected drug is topotecan as its active moiety of lactone operating during the S phase of the cell cycle, is commonly used in combination with melphalan to take advantage of synergistic effects (Kaufmann et al., 1996; Schaiquevich et al., 2012b).

7.1.2.1. Intraocular distribution of intra-arterial melphalan and topotecan

Pharmacokinetics studies after intra-arterial melphalan in non-tumor bearing pigs, found that attained vitreous C_{max} barely reached the concentration that inhibits 50% of the retinoblastoma cell growth *in vitro* (IC₅₀), whereas the vitreous AUC was 3-fold higher than the plasma AUC (Schaiquevich et al., 2012b) (Fig 19). The relatively low intravitreal levels were thought to be related to a poor affinity of the melphalan to the L-amino acid transporter 1 (LAT1), the mediator of its transport through the blood-retina barrier (Hosoya et al., 2008). Comparatively, intravitreal concentrations of topotecan after intra-arterial injection using the same animal model were above IC₅₀ for at least 4 hours (Schaiquevich et al., 2012a) (Fig 20) and the AUC in the vitreous was 29-fold higher than that in the bloodstream of the study pigs (Schaiquevich et al., 2012a). Another study also performed in pigs showed 143- and 246-fold higher retina and vitreous levels respectively after intra-arterial topotecan than after intravenous infusion of the

same dose (Taich et al., 2016), with comparable systemic exposure. In the same study, topotecan exposure in the optic nerve was 80-fold higher after intra-arterial injection than after intravenous infusion at the same dose of 4 mg.

Despite the melphalan vitreous-to-plasma ratio of almost 10-fold less than the ratio obtained for topotecan in non-tumor pigs studies (Schaiquevich et al., 2012a; Schaiquevich et al., 2012b), it was clear from clinical experience that melphalan alone was efficient to treat retinal and subretinal tumors, whereas this was not the case with intra-arterial topotecan as monotherapy (see 7.2.2). On the other hand, intra-arterial melphalan, alone or in combination with topotecan, was insufficient to manage all cases with vitreous disease.

Of note, melphalan was detected in the RPE-choroid in almost all the eyes of the pigs after intra-arterial chemotherapy but barely quantified in the retina, showing the selective accumulation of the drug in the RPE-choroid tissue.

7.1.2.2. Systemic adverse effects of intra-arterial delivered drugs

Although intra-arterial chemotherapy is a local route for ocular drug delivery, a fraction of the administered drug becomes systemically available. This implies that there is a limitation to the maximum dose that can be given in order to avoid systemic adverse effects, and supplementary caution should be made in cases of tandem therapy. Normalizing the dose based on body weight was found to minimize inter-patient variability in melphalan pharmacokinetics. Thus, patients exceeding the threshold dosage of 0.48 mg/kg showed a 50% chance of presenting severe neutropenia compared to less than 1% in children receiving lower dosages (Dunkel et al., 2014; Schaiquevich et al., 2012b).

Finally, a clinical pharmacokinetic study on intra-arterial chemotherapy with combined melphalan (0.5 mg/kg) and topotecan (0.5-1 mg) in patients with retinoblastoma, failed to show

severe hematologic toxicity secondary to a potential drug-drug interaction (Taich et al., 2014). Total topotecan systemic exposure after intra-arterial chemotherapy was 95 ng*h/ml per mg of topotecan with about one-third of this exposure corresponding to the active lactone moiety (Taich et al., 2014), which is way below the threshold for severe neutropenia after intravenous delivery (Brennan et al., 2017).

7.1.3. Intravitreal drug delivery

Compared to all the others administration routes, drug injection directly into the vitreous bypasses the ocular barriers and the first-pass elimination, and provides therefore the highest intraocular exposure in the vitreous humor, ideal for the management of vitreous disease. After the intravitreal injection, elimination takes place anteriorly by drug flow from the vitreous into the aqueous humor and thereafter Schlemm's canal and posteriorly through the components of the blood-retinal barriers to the choroidal blood flow and the systemic circulation.

Similar to intra-arterial chemotherapy, the most commonly injected drugs are melphalan and topotecan. Other drugs, such as carboplatin and etoposide phosphate, are under investigation in preclinical studies with rabbits as a potential alternative to melphalan, but will not be discussed here as they are not currently used clinically (Mohny et al., 2017).

7.1.3.1. Intraocular distribution of intravitreal melphalan

Intravitreal melphalan (15 µg-dose) in rabbits resulted in vitreous concentrations above the IC₅₀ for up to 5 hours, while undetectable in plasma throughout the 12-hour period of the study (Buitrago et al., 2016) (Fig 19). The high selectivity for the vitreous and the low systemic exposure of melphalan after intravitreal injection in the animal study are in agreement with the notable efficacy for vitreous seeds control and the lack of hematologic toxicity reported in patients treated this way (see 7.2.3). On the other hand, the drug aqueous humor exposure was

less than 1% of that in the vitreous, making it inadequate for targeting anterior chamber seeding (Munier et al., 2017a).

Although data are limited, binding of drugs to vitreous proteins as well as drug interaction with melanin has been hypothesized to lead to prolonged residence time in the vitreous humor and pigmented tissues (iris, ciliary body, retinal pigment epithelium, choroid) respectively, resulting in prolonged pharmacological toxicity (Rimpela et al., 2017). This may explain, at least in part, the retinal and iris toxicity reported after intravitreal melphalan in patients or in a preclinical rabbit model, as well as the greater reduction in electroretinogram (ERG) components observed in more pigmented human eyes (Francis et al., 2015b; Francis et al., 2017b; Francis et al., 2014).

7.1.3.2. Intraocular distribution of intravitreal topotecan

In rabbits, the intravitreal administration of only 5 μ g of topotecan led to vitreous concentrations above the IC₅₀ for up to 16 hours after the injection (Buitrago et al., 2010), with no retinal toxicity (Buitrago et al., 2013). These pharmacologically active concentrations after such low doses have never been attained with other routes of ocular delivery. In addition, the selectivity of this route was demonstrated by a vitreous-to-plasma ratio of 57 (Fig 20). After intravitreal injection, topotecan exposure in the aqueous humor was, however, only 5% of that in the vitreous and that only in its carboxylate form, considered to be pharmacologically inactive, whereas the active lactone form was undetectable (Buitrago et al., 2010). Like intravitreal melphalan, this makes it inappropriate to treat aqueous seeding.

7.1.4. Intracameral drug delivery

Intracameral injections were specifically developed to target the anterior and posterior chambers as aqueous seeding had remained untreatable with other delivery routes (see 7.2.4).

Thus, despite drug penetration to the anterior chamber after intravenous (see 7.1.1) or periocular delivery (see 7.1.5) achieving an even higher aqueous C_{max} concentration compared to the intravitreal one in both human and animal eyes (see 7.1.3), the obtained doses were not high enough to reach tumoricidal levels. On the other hand, neither intravitreal melphalan (Buitrago et al., 2016) nor topotecan (Buitrago et al., 2010) achieved pharmacologically active levels in the aqueous humor (see 7.1.3) in a non-tumor bearing rabbit model. Finally, although preclinical studies have not been designed to study aqueous concentrations after intra-arterial chemotherapy, the latter has never been reported to be successful in the management of aqueous seeding (see 7.2.4).

To date, very limited data are available on the drug pharmacokinetics after intracameral injections, but clinical evidence showed them to be successful in treating aqueous seeding, although insufficient to control intravitreal disease (Munier et al., 2015; Munier et al., 2017a).

7.1.5. Periocular drug delivery

Periocular delivery was developed before the use of intra-arterial and intravitreal chemotherapy in an attempt to increase the intraocular bioavailability of the chemotherapeutic drugs. After periocular injection, the intraocular bioavailability is limited by the sclera, choroidal and outer blood-retinal barrier, as well as by the orbital and choroidal clearance (Del Amo et al., 2017; Edelhauser et al., 2010). The diffusion of periocular injected drugs can be stimulated by transcleral iontophoresis, as shown for low-molecular-weight charged molecules, such as carboplatin (Hayden et al., 2006).

Preclinical studies in rabbits showed a 6-fold increase in vitreous-to-plasma AUC after periocular carboplatin compared to the same ratio after intravenous infusion (Hayden et al.,

2004; Simpson et al., 2002) and, in the non-human primate, the vitreous and aqueous humor C_{\max} was over 2-fold the levels attained in plasma (Mendelsohn et al., 1998).

Similar studies with periocular topotecan showed vitreous drug exposure of only 30% of the systemic AUC after periocular injection in rabbits (Carcaboso et al., 2007) (Fig 19), whereas in non-tumor-bearing rats and pigs, topotecan vitreous AUC was 2- and 3-fold the systemic exposure, respectively (Nemeth et al., 2011; Schaiquevich et al., 2012a), possibly due to inter-species differences in the orbital and ocular vasculature. Lastly, pharmacokinetic data on topotecan after periocular delivery in humans obtained from a Phase I study in retinoblastoma patients, revealed a non-toxic systemic exposure to lactone topotecan below 55 ng/ml/h (Chantada et al., 2009).

7.1.6. Suprachoroidal drug delivery

Despite not being explored for targeting retinal diseases, suprachoroidal administration may provide an alternative route for drug delivery to the posterior segment. The drug is placed in the space between the sclera and choroid and could be used to localize drug delivery to the choroid, retinal pigment epithelium and, to a lesser extent, the retina (Patel et al., 2011). A rapid diffusion of the injected solution around the eye has been shown in animal models (Chiang et al., 2018). An interesting aspect is the use of microneedles for performing the injection that allows minimal damage of the sclera and avoids penetration into the choroid and retina (Hartman and Kompella, 2018; Patel et al., 2011).

Although suprachoroidal drug delivery may be advantageous for targeting the choroid and retinal pigment epithelium, less evidence is available to demonstrate adequate and prolonged exposure in the retina (Chiang et al., 2018; Del Amo et al., 2017).

7.2. Treatment modalities

Retinoblastoma has always been credited with the highest 5-year disease-free survival rate among pediatric cancers, presently reaching 97-99% in high-income countries (Fernandes et al., 2018; Group et al., 2013; Jenkinson, 2015). Until the recent introduction of new targeted treatment modalities, namely intra-arterial and intravitreal chemotherapy, survival was often only achieved using mutilating and disabling therapies. During the irradiation era, 96% of bilaterally affected children had their worst eye enucleated at diagnosis, with primary enucleation of both eyes necessary in 3% and secondary enucleation of an only remaining eye due to treatment failure leading to total blindness in 25-29% (Abramson et al., 2004; Epstein et al., 2003). Despite the advent of systemic chemotherapy as first line treatment, 64-66% of children with bilateral disease still underwent primary enucleation of the more advanced eye and 2-6% required initial bilateral enucleation (Epstein et al., 2003; Kim et al., 2008), while advanced diseased eyes frequently needed external irradiation and/or secondary enucleation. The recent advances in targeted chemotherapy modalities, allowed treatment of advanced disease and salvage of heavily pre-treated eyes, resulting in a further decline of the primary enucleation rate to less than 8% and virtually no more bilateral ocular losses (Francis et al., 2018b). A similar preference for primary conservative treatment can be observed in unilateral disease with a continuing decrease of primary enucleation, from 95% of the cases during the late 20th century, to 56% in the early 21st century and only to 5-7% currently (Abramson et al., 2015a; Munier et al., 2017b). This marked improvement in globe salvage, without the need of external beam irradiation, undeniably contributes not only to a better quality of life, but is also expected to reduce the incidence of binocular visual impairment and blindness in this population (Stacey et al., 2019).

In Lausanne, the simultaneous availability since 2008 of both intra-arterial and intravitreal chemotherapy as additional options to the traditional chemoreduction and focal treatment, contributed to achieve 5-year ocular survival estimates of almost 100% for treatment-naïve IIRC group A-C (98%, 99% CI 86.7%-99.7%) (Fig 21), and significantly improved the ocular survival of treatment-naïve IIRC group D and E eyes, with Kaplan-Meier estimates of 85.7% (95%CI 75.7%-91.8%) at 2-years and 79.4% (95% CI 67.0%-87.6%) at 5-years (very similar to the 80.2% for COG group D and E after Abramson (Abramson et al., 2017a)), with no metastasis in the cohort of naïve cases. This compares favorably to the era prior to 2008, where ocular survival estimates were 55.0%, (95% CI 38.7%-68.7%) at 2- and 49.2% (95% CI 33.0-63.6%) at 5-years ($p=0.0007$) (Fig 22).

7.2.1. Intravenous chemotherapy

The indications for intravenous chemotherapy in the treatment of retinoblastoma have progressively changed over the years. During the era of external beam radiotherapy (Ellsworth, 1969), systemic chemotherapy was limited to extraocular (Doz et al., 1995; Pratt et al., 1985) or metastatic disease (Saleh et al., 1988). In the 1990s, its role extended to first line treatment with the concept of chemoreduction, aiming to render the tumor volume amenable to focal treatment (Greenwald and Strauss, 1996; Kingston et al., 1996; Murphree et al., 1996; Shields et al., 1996). Systemic chemotherapy contributed to increase globe salvage rate, significantly reducing the need for external beam radiotherapy and the incidence of secondary primary malignancies. To date, systemic chemotherapy has been supplanted in part by the introduction of intra-arterial (see 7.2.2) and intravitreal (see 7.2.3) chemotherapy (Abramson et al., 2015b).

7.2.1.1. Drug regimen and number of cycles

Initiation of systemic chemotherapy usually takes place within 24 to 48 hours after the first ophthalmological examination to take advantage of the focal cryorupture performed to improve drug penetration into the vitreous and/or subretinal fluid (Wilson et al., 1996). Before each new chemotherapy cycle, the child undergoes an examination under anesthesia in order to evaluate the tumor response. After completion of the second course of chemotherapy, sequential intensive focal treatment is applied for every lesion that is not flat or calcified (Murphree et al., 1996) (see 7.2.6).

The decision regarding the drug combination and number of courses needed has remained highly variable across different referral centers. The majority of studies report the use of 6 courses of a 3-drug regimen (carboplatin-etoposide-vincristine) combined with focal treatments (Table 9). Different observations corroborate the approach, however, that a 2-drug regimen (etoposide-carboplatin) with a limited number of courses (2 to 3) could be considered as the standard of intravenous chemotherapy for intraocular retinoblastoma, whereas other therapeutic options have to be employed depending on disease evolution, as no long-term benefit of prolonged systemic chemotherapy is obtained. Indeed, systemic chemotherapy alone (without consolidation) has been reported to reduce tumor volume by approximately 35% diameter and 50% thickness already after the two first cycles (Shields et al., 1996). Absence of further reduction of tumor thickness with additional course of intravenous chemotherapy is further demonstrated by the data presented in Figure 23 (Lausanne, unpublished data). In addition, compared to groups giving 6 or more cycles of a 3-drug regimen, equivalent tumor control was achieved with a mean of 3 cycles of a 2-drug regimen (etoposide-carboplatin) (Beck et al., 2000), even for group D eyes (Munier et al., 2017b). Finally, in another study evaluating the efficacy

of 3 versus 6 cycles of carboplatin, etoposide, vincristine for group B eyes with dose escalation and additional cycles in the short course chemoreduction group if needed, no difference in the globe salvage rate was found between the two groups at 5-year follow-up, sparing 63% of the patients treated with the short course approach the extra 3 cycles of systemic chemotherapy (Zhu et al., 2015).

7.2.1.2. Indications

7.2.1.2.1. Current indications

In the era of intra-arterial chemotherapy, systemic chemotherapy remains the treatment of choice for all tumors not accessible to focal treatments in children younger than 3 months of age and/or weighing less than 5 kg, until there is sufficient vascular maturation to proceed safely with the catheterization of the ophthalmic artery (so-called bridge therapy) (Gobin et al., 2012). Systemic chemotherapy can also be used in the rare cases of technical failure to catheterize the ophthalmic artery, or as salvage treatment with a 2 or 3-drug regimen for recurrent disease resistant to intra-arterial chemotherapy.

Advanced bilateral cases are treated with systemic chemotherapy in some centers, while others perform tandem intra-arterial chemotherapy (Abramson et al., 2015b). The indications for asymmetric bilateral cases with one eye accessible to focal treatment and unilateral cases, including advanced group IIRC D and selected E eyes, have been now replaced by intra-arterial, combined or not with intravitreal chemotherapy (Abramson et al., 2015b) (see 7.2.2).

7.2.1.2.2. Potential future indications

One case of choroidal relapse that regressed totally to a flat scar already three weeks after the first cycle of high-dose chemotherapy given for concomitant trilateral disease has been recently reported (Stathopoulos et al., 2018b). The long-term efficacy of systemic chemotherapy in such a

situation remains however unknown, as the child died of intravascular disseminated coagulation-like disease after the 5th systemic chemotherapy in her country of origin.

Unlike chemoreduction, intra-arterial chemotherapy appears to be associated with a significant risk of rhegmatogenous detachment affecting specifically endophytic eyes in 1/4 (Shields et al., 2017) to 1/3 of the cases (FLM unpublished data) (see 7.3.4.1). This complication may be explained by the faster tumor shrinkage observed with intra-arterial chemotherapy, inducing sudden shearing forces and consecutive dehiscence at the retino-tumoral junction. A single course of chemoreduction given prior further intra-arterial chemotherapy may be beneficial to reduce the incidence of rhegmatogenous retinal detachment in advanced endophytic retinoblastoma.

7.2.1.3. Treatment outcomes

7.2.1.3.1. Relapse rate and role of consolidation by focal treatments

Since its introduction in the conservative treatment of retinoblastoma, systemic chemotherapy was commonly used in combination with focal treatment by most groups (see Table 9). The role of consolidation was specifically pointed out in a study which demonstrated that chemotherapy alone (without consolidation) failed complete inactivation of the disease in 92% of treated eyes, even after completion of 6 cycles (Wilson et al., 2001). When retinal tumors were treated by combined chemoreduction and focal therapy, disease control was shown to improve substantially ($p=0.05$), with a 2.5 reduction of the relapse risk (Shields et al., 2004b). In addition, focal treatment given following chemoreduction as prophylactic consolidation (Wilson et al., 2005) rather than deferred until documented disease progression (Rodriguez-Galindo et al., 2003) was credited to decrease the need for external beam radiotherapy for salvage after chemoreduction from 44 to 25-30% (Wilson et al., 2005).

7.2.1.3.2. *Eye preservation without external beam radiotherapy*

Despite the concomitant use of diverse grouping classifications, and the variety of protocols in terms of chemotherapy regimen and number of cycles making comparison between studies difficult, major studies with at least 2-year mean follow-up claim an overall tumor control of 70-100% for RE I to III or IIRC group A to C and 23-64% for RE IV/V or IIRC group D/E (Table 9). Despite an unsatisfactory outcome in advanced eyes, intravenous chemotherapy contributed, however, overall to a significant decrease in the use of irradiation and consequently the incidence of secondary radiation-induced tumors (see 3.8).

7.2.1.4. *Chemotherapy-related toxicity*

Chemotherapy-related toxicity is one of the major concerns in the treatment of pediatric cancers. Even if common and relatively easily managed in centers of high-income countries, chemotherapy-related adverse effects may sometimes result in fatal toxicities in less developed countries (Luna-Fineman et al., 2018).

7.2.1.4.1. *Expected toxicities*

Systemic chemoreduction with standard carboplatin-etoposide±vincristine regimens usually causes mild acute toxicity, mostly involving alopecia, nausea/vomiting, and moderate myelotoxicity, occasionally requiring hospitalization for fever, neutropenia or blood product transfusions. The use of more intense regimens is predictably associated with higher myelotoxicity. Febrile neutropenia requiring hospitalization has been variably reported in 12.7-38% of the patients, depending on the specific chemotherapeutic regimen (Bartuma et al., 2014; Berry et al., 2013; Chung et al., 2008; Kunkele et al., 2013). The need for transfusion of blood products is also inconsistently reported, ranging from 0% (Berry et al., 2015) to 78% (Brennan et al., 2017) of the patients depending on the intensity of treatment. Support with Granulocyte-

colony stimulating factor was used by some groups for 100% of the patients (Brennan et al., 2017), whereas others completely avoided its use (Chantada et al., 2014).

7.2.1.4.2. Long-Term toxicities

As previously discussed (see 3.8.3.2.2), intravenous etoposide may induce secondary leukemias, even in patients who received what are considered safe cumulative dosages (Chantada et al., 2014; Gombos et al., 2007). Permanent ototoxicity is an important late effect of carboplatin-based chemoreduction regimens reported in up to 5-25% of the cases, especially if given at a younger age and with prolonged exposure (Jehanne et al., 2009; Qaddoumi et al., 2012; Soliman et al., 2018).

7.2.2. Intra-arterial chemotherapy

The success of intra-arterial chemotherapy lies in the selective delivery of high dose drug(s) to the eye with minimal systemic absorption. The modern technique of drug injection directly into the ophthalmic artery through a trans-femoral microcatheter placed just beyond its ostium, also called super-selective intra-arterial chemotherapy or ophthalmic artery chemosurgery, was introduced in 2008 by Abramson et al. (Abramson et al., 2008), inspired by previous work by Kaneko et al. (Kaneko and Takayama J et al, 1990) and Yamane et al. (Yamane et al., 2004).

In the hands of well-trained interventional neuro-radiologists, the procedure is safe and reaches a technical success rate of around 96-99% (Francis et al., 2018a; Munier et al., 2011; Suzuki et al., 2011), provided that it is performed in children with sufficient vascular maturation (children older than 3 months and with a body weight of over 5kg) (Gobin et al., 2012). Anatomic variants such as departure of the ophthalmic artery from the meningeal artery, or unfavorable angle with the internal carotid artery compromising direct access to the ophthalmic artery for the delivery of the chemotherapy (around 16% of the procedures) (Abramson et al.,

2012a), can usually be overcome with the use of alternative non-ophthalmic routes and/or of a temporary balloon-occlusion of the internal carotid (Klufas et al., 2012). When assessing treatment response one should be aware that anastomoses between the external and internal carotid or stenosis of its ostium, as a consequence of a vasospasm or micro-dissection from a previous procedure, may alter the local hemodynamic flow of the ophthalmic artery, and lead to insufficient drug perfusions (Francis et al., 2018a).

7.2.2.1. Drug regimen and number of cycles

There is no consensus to date either on the minimal drug dose required, or on any advantage to start the treatment with a single versus a combination of drugs. The drug most commonly used is melphalan. Topotecan and/or carboplatin can be used in combination with melphalan (2 or 3-drug regimen) to capitalize on their synergistic effects. One to 3 cycles of melphalan are generally given on a 3 to 4-week basis in intra-arterial chemotherapy-naïve eyes, and a combination of drugs (usually melphalan and topotecan) for tumor relapse or poor response following previous treatment with melphalan only.

Carboplatin is usually given to minimize systemic side-effects in the less advanced eye when combined (or so-called tandem) intra-arterial chemotherapy is performed. When doing so, the more advanced eye is usually treated with melphalan (with or without topotecan). Drugs between the eyes are then switched during the second or third session (Abramson et al., 2010; Abramson et al., 2016b).

7.2.2.2. Indications

7.2.2.2.1. Current indications

Initially used as salvage treatment for eyes that did not resolve with systemic chemotherapy and/or radiotherapy, indications for intra-arterial chemotherapy extended to first line treatment

for naïve unilateral eyes (group B with macula and/or papilla involvement, and group C and D). Other indications include the more affected eye in bilateral cases with asymmetric presentation, provided that the fellow eye is accessible to focal therapy. Simultaneous tandem therapy is performed by some groups for bilateral cases with any combination of group B with macula/papilla involvement, C, D or selected E eyes, namely those characterized by tumor touching the lens with or without total retinal detachment (Abramson et al., 2015b; Rowlands et al., 2018) (Table 10), whereas some centers prefer to start with chemoreduction, followed by intra-arterial chemotherapy if needed for salvage treatment (Abramson et al., 2015b).

Group E eyes with neovascular glaucoma, buphthalmos, orbital cellulitis, anterior chamber invasion or phthisis undergo enucleation. In cases where enucleation cannot be performed safely, neoadjuvant systemic chemotherapy can be advised prior to surgery, especially in eyes with buphthalmos and/or radiological evidence of optic nerve invasion (Bellaton et al., 2003).

7.2.2.2.2. *Potential future indications*

Intra-arterial chemotherapy with combined melphalan and topotecan was reported to successfully treat one case of a choroidal relapse that showed complete regression on UBM three weeks after the first injection. The child received two more intra-arterial injections and has since then remained metastasis- and recurrence-free at an updated follow-up of more than 4 years (Stathopoulos et al., 2018b). In another recent report, two courses of intra-arterial melphalan enabled to control iris invasion in a monophthalmic patient, but not a concomitant intra-ciliary involvement responding only to brachytherapy with an updated disease-free follow-up of more than 20 months (Munier et al., 2018).

The differential efficacy of intra-arterial chemotherapy on iris and choroid vs ciliary body may be explained by the lower vascular supply of the latter consisting of 2 long posterior ciliary

arteries vs 7 anterior and 20 short posterior ciliary arteries vascularizing iris and choroid respectively.

7.2.2.3. Treatment outcomes

Treatment outcomes of the major studies obtained with first-line and salvage intra-arterial chemotherapy are summarized in Table 11.

7.2.2.3.1. Recurrence rate and salvage therapy

Intra-arterial chemotherapy appears to be associated with an increased risk of recurrence if administered more than 4 weeks apart ($p < 0.05$) and if more than 50% of the injections are delivered via a non-ophthalmic route of delivery ($p < 0.001$) (Francis et al., 2018a). The role of focal consolidation remains to be determined. Recurrences after first line or salvage intra-arterial chemotherapy have been observed within the first year following treatment completion in 24-25% of the cases receiving also focal consolidation (Francis et al., 2018a; Munier et al., 2017b), and in 29% of those without consolidation (Tuncer et al., 2016).

Relapse after intra-arterial chemotherapy can be successfully managed with focal treatments, including intravitreal chemotherapy in more than half of the cases (Francis et al., 2018a; Munier et al., 2017b). A second course of intra-arterial chemotherapy can, however also be given if needed, after first-line or salvage intra-arterial chemotherapy, with a reported globe salvage rate of about 76% (Francis et al., 2015a; Shields et al., 2015).

7.2.2.3.2. Eye preservation without external beam radiotherapy

Comparison of the studies on globe salvage rate with intra-arterial chemotherapy is not only challenged by the lack of randomized clinical trials and the use of different versions of the retinoblastoma classification (see 3.3.1), but also by the availability of intravitreal chemotherapy at the time of the treatment. A survey of the literature during the four years where only intra-

arterial was available, i.e. before the 2012 publication of the safety enhanced technique for intravitreal chemotherapy, reveals that intra-arterial chemotherapy as first line treatment achieved globe salvage without the need of external beam irradiation in 96% group B and C eyes at a median follow-up of 16 months (Abramson et al., 2012a), 81% at 2 years of group D (Reese-Ellsworth V) (Gobin et al., 2011), but only 64% at 2 years for those with vitreous seeding (including group C, D and E) (Abramson et al., 2012c).

After 2012, first line intra-arterial chemotherapy associated with intravitreal chemotherapy as necessary, achieved globe salvage without the need of external beam irradiation in 100% ICRB/IIRC group B and C at a mean follow-up of 19 months (Shields et al., 2014), a 5 year-ocular survival of 85-100% COG/IIRC group D (Abramson et al., 2017a; Munier et al., 2017b), and 36 in ICRB group E at a mean follow-up of 19 months (Shields et al., 2014).

7.2.2.4. Adverse effects

Intra-arterial chemotherapy is safe, with no reported fatal complications and fewer systemic adverse effects compared to intravenous chemotherapy and external beam irradiation. Only one case of delayed neurological complication that spontaneously resolved has been described to date (De la Huerta et al., 2016). An intra-operative autonomous reflex characterized by hypoxia, reduced lung compliance, systemic hypotension and bradycardia, that can be easily managed by an experienced anesthesiologist, develops in about 24% of the internal carotid catheterization procedures (Phillips et al., 2013). In the post-operative period, some degree of neutropenia (grade 1 or 2) is the most frequently reported systemic side effect after intra-arterial melphalan (about 12% of the cases), but usually remains subclinical if the melphalan dose does not exceed 0.4-0.5mg/kg (Dunkel et al., 2014). In the context of tandem injections, however, the occurrence of clinically significant fever/neutropenia was described in 1% (2/209) of the procedures and

transfusion of blood product was limited to 4 transfusions in three patients (1.9% of the events) (Abramson et al., 2016b). Evaluation of the immune function in retinoblastoma patients treated with intra-arterial chemotherapy failed to detect the presence of a clinically significant immune suppression, justifying pneumocystis pneumonia prophylaxis (Fischer et al., 2017).

Locally, adverse effects consist of redness (29%), transient eyelid edema (5%), loss of eyelashes (5%), blepharoptosis (5%), forehead hyperemia (2%), and rarely third or sixth nerve palsy, each of them resolving within 2 to 3 months (Wyse et al., 2016). Intraocular vascular complications, such as ophthalmic artery obstruction (2%), vitreous hemorrhage (2%), choroidal occlusive vasculopathy (7%), are of greater concern (see 7.3.7), especially in eyes with visual potential, as they can irreversibly compromise vision (Dalvin et al., 2018; Shields et al., 2014).

7.2.3. Intravitreal chemotherapy

The concept of intravitreal chemotherapy was confined for decades to isolated heroic attempts with thiotepea in desperate cases (Ericson and Rosengren, 1961; Seregard et al., 1995), due to the risk of extra-ocular tumor spread. In 1994, Akihiro Kaneko started using this route of drug delivery, injecting 8 µg of melphalan combined with whole-eye hyperthermia for vitreous disease and reported a 51.3% eye survival rate at more than 50 months follow-up (Kaneko and Suzuki, 2003), comparable to the results achieved with external beam radiotherapy (Abramson and Scheffer, 2004b) and chemoreduction (47%) (Shields et al., 2002). After the introduction of intra-arterial chemotherapy in 2008, the probability of globe preservation in eyes with vitreous seeding increased to 64% in treatment-naïve eyes at 2 years follow-up, but enucleation was still necessary in about 30% of the eyes due to uncontrollable vitreous disease (Abramson et al., 2012c). Similarly, in 2011, Shields et al. published a 67% control of the vitreous disease at approximately one year follow-up (Shields et al., 2011).

In 2012, the development of a safety-enhanced technique for intravitreal injection through a tumor-free pars plana site using anti-reflux measures and needle tract sterilization (Munier et al., 2012b) enabled vitreous disease to be specifically addressed and became rapidly adopted worldwide, leading not only to the reduction of the enucleation rate, but also definitively eradicating the need for external beam irradiation. Since then, long-term studies have confirmed safety and efficacy for both primary and recurrent vitreous seeding, approaching 100% of vitreous disease control (see 7.2.3.3). On the other hand, clinical observations and a better knowledge of the cellular composition of the seeding allowed us to re-evaluate the treatment response and redefine the treatment intensity according to the seed classification. Finally, increased efforts have been made to understand and minimize intravitreal melphalan-related toxicity (see 7.3.6.1).

7.2.3.1. Drug regimen and number of injections

As for intra-arterial chemotherapy, the most commonly used drug for intravitreal injections is melphalan. Preclinical studies have established that melphalan of at least 4 $\mu\text{g/ml}$ is required to fully suppress retinoblastoma cells *in vitro* (Inomata and Kaneko, 1987), and that at a vitreous concentration of 5.9 $\mu\text{g/ml}$ is functionally and structurally non-toxic to the retina of albino rabbits (Ueda et al., 1995). To date, there is no international consensus regarding the starting dose, but we recommend 20 to 30 μg depending on eye size and tumor volume (see 7.3.6.3). A lower dose (8 μg) has been reported to be ineffective, whereas a higher dose (50 μg) was associated with phthisis bulbi (Ghassemi and Shields, 2012).

The second most frequently injected drug is topotecan, usually combined with melphalan in case of failure of tumor control, potentially due to melphalan resistance, or recurrences. Noteworthy, in the 10-year experience with intravitreal melphalan in Lausanne (more than 160

eyes and 750 injections), there was only a single case of true resistance to melphalan requiring topotecan to control the recurrent vitreous disease (personal observation). Compared to melphalan, topotecan has a longer intra-ocular half-life, with low ocular toxicity and also the advantage of a lower cost (Schaiquevich et al., 2017).

Intravitreal chemotherapy is carried out every 7-10 days. At each visit, the residual vitreous tumor burden is reassessed and usually an additional injection for consolidation is given once complete regression is observed. Complete response is established if the seeds either completely disappear (vitreous seeding regression type 0) or convert into refringent and/or calcified residues (vitreous seeding regression type I), amorphous often non-spherical inactive residues (vitreous seeding regression type II), or a combination of the latter two (vitreous seeding regression type III) (Munier, 2014). Another useful criterion of complete response is the presence of hyper-reflective dots within class 2 seeds as shown by OCT (see 3.2.3) (Munier, 2014). The timing to complete regression is a matter of weeks, months and up to one year for class 1, 2 and 3 seeds respectively (Table 12). Since the initial recommendations (Francis et al., 2015a), the number of injections necessary to achieve tumor control for the various classes of vitreous seeds can be reduced, namely two for class 1, three for class 2, and four for class 3.

7.2.3.2. *Safety*

Since the adoption of the safety-enhanced technique of injection in 2012, there has been no report, to our knowledge, of any tumor exteriorization as documented by a meta-analysis of the published literature (Smith and Smith, 2013) or by retrospective analysis of more than 3500 injections performed in 10 North and South American, European, Israeli, and Chinese centers (Francis et al., 2017a). More recently, the safety of intravitreal chemotherapy was further confirmed when assessing the expression of the cone-rod homeobox gene (CRX) by real-time

quantitative polymerase chain reaction as a surrogate of retinoblastoma RNA, failed to detect any CRX-positive cycle from the entry site or from the cryoprobe tip (Winter et al., 2018).

7.2.3.3. Treatment outcomes

Intravitreal melphalan is efficient with a reported vitreous seeding control in 69-100% of the cases (Table 13). Two single-institution studies have compared the outcomes in the eras before and after the introduction of intravitreal chemotherapy. In the first, complete abandon of external beam radiotherapy was effective only after the introduction of intravitreal chemotherapy (Francis et al., 2018a), while the second documented a significant improvement of ocular survival from 77 to 87%, and from 25 to 73% in ICRB group D and E eyes respectively ($p<0.04$) (Shields et al., 2016a), as well as a threefold decrease in the enucleation rate during the pre-intravitreal (44%) vs intravitreal (15%) era ($p=0.012$). In a study comparing the outcome of eyes with class 3 seeding (cloud) treated with intra-arterial and intravitreal chemotherapy versus intra-arterial alone, the authors demonstrated a significantly shorter time to regression (5.7 months vs 14.6 months, $p<0.001$) as well as significantly fewer recurrences and fewer enucleations with the concomitant use of intravitreal chemotherapy (Francis et al., 2017c). The same group also reported on their 4-year experience with intravitreal chemotherapy in 130 eyes with a 2-year Kaplan-Meier estimate for ocular survival of 94.2% (CI 89.2-99.4%), and an estimate for disease-free survival of 86.2% (Francis et al., 2017b). According to the literature, there also seems to be no significant difference between eyes with persistent versus recurrent vitreous seeding regarding time to regression, number of injections needed, or cumulative dose of melphalan (Berry et al., 2017a; Ji et al., 2016; Kaneko and Suzuki, 2003; Munier et al., 2012a).

Until now, additional topotecan was usually considered in the case of resistant vitreous seeding and injected with concomitant melphalan (Francis et al., 2017b; Ghassemi et al., 2014;

Shields et al., 2016b). In a recent paper, control of vitreous disease was achieved with intravitreal topotecan alone in 100% of the case (n=17) at a median follow-up of 24 months with no clinical ocular toxicity (Rao et al., 2018b).

Finally, although intravitreal chemotherapy is not primarily directed against retinal or subretinal tumors, partial or complete regression of subretinal seeds after intravitreal melphalan has been documented in isolated cases (Francis et al., 2015c; Munier, 2014; Shields et al., 2016b). The efficacy of intravitreal chemotherapy for indications other than active vitreous disease remains, however, to be established (Abramson et al., 2018a; Abramson et al., 2018b).

7.2.3.4. Adverse effects

Intravitreal injections of melphalan have proved to have fewer adverse effects and no systemic toxicity compared to intra-arterial chemotherapy (Francis et al., 2014). The most common side effect after standard doses of intravitreal melphalan is localized salt-and-pepper retinopathy at the site of injection, reported in 0.8-43% of the cases (Smith et al., 2014), which significantly correlates with reduction in ERG amplitudes with an estimated 5.3 μ V decrease for every intravitreal melphalan injection of 25-30 μ g (Francis et al., 2017b) (see 7.3.6.1). Other rare side effects include sub-conjunctival hemorrhage, mild vitreous hemorrhage, cataract, transient hypotony and retinal detachment (Smith et al., 2014). Iris depigmentation, iris recession with retinal necrosis and focal scleromalacia with localized pigmentation have been reported in isolated cases (Francis et al., 2015c).

7.2.4. Intracameral chemotherapy

Aqueous seeding (see 6.2.4.1) with or without concomitant anterior uveal involvement was until recently considered an intractable form of retinoblastoma. The prognostic value of aqueous seeding and/or anterior uveal invasion such as iris or corpus ciliaris is controversial, but

is no longer considered to be high risk for metastasis and, consequently, does not represent an automatic indication for adjuvant chemotherapy (Baroni et al., 2014; Sreelakshmi et al., 2017; Suryawanshi et al., 2011).

Along the same lines as for intravitreal injections, a safety-enhanced technique was developed for intracameral injections and used for the first time in 2011 to successfully treat aqueous seeding in a patient with diffuse anterior retinoblastoma (Munier et al., 2015; Munier et al., 2017a). Unlike vitreous volume, the exact value of aqueous volume according to age is not known, which precludes the precise determination of the aqueous drug concentration following the injection of a given dose. In order to obtain in all injected eyes the same final aqueous concentration of melphalan irrespective to age, athalamia is first created by a paracentesis emptying both anterior and posterior chambers, followed by the reconstitution of the cameral volume by injecting a solution at the desired final melphalan concentration (15-20 $\mu\text{g/ml}$) (Munier et al., 2017a).

Alternatively, it is also possible to inject very small volumes of highly concentrated drug without prior anterior chamber tap, i.e. 0.015 ml at 500 $\mu\text{g/ml}$ (7.5 μg) of topotecan (Paez-Escamilla et al., 2017), or 0.05 ml at 30 $\mu\text{g/ml}$ (15 μg) of melphalan (Cassoux et al., 2017). This technique, however, is suboptimal regarding the drug concentration achieved in the aqueous humor, with a risk of underexposure at the level of the posterior chamber.

7.2.4.1. Treatment outcomes

Following the first case report on intracameral chemotherapy (Munier et al., 2017a), a series of 11 eyes with secondary aqueous seeding treated with this new technique was published (Munier et al., 2018). In this study, intracameral melphalan injection contributed to a globe preservation of 55% of the treated eyes at a mean updated event-free follow-up of 29 months

(range 16-48), including 5 only eyes. Despite initial complete regression of the aqueous seeds, as assessed by cytopathology and cell culture (Munier et al., 2018), five cases were nevertheless enucleated. Overall, the eye retention rate appeared to be less favorable in eyes with iatrogenic disrupted anterior hyaloid (25% or 1/4, salvaged), compared to eyes with intact anterior hyaloid (71% or 5/7 salvaged). Since this publication, three additional patients with anterior chamber seeding benefited from intracameral chemotherapy in Lausanne, with a recurrence-free follow-up of 3, 6, and 14 months respectively (personal observation).

7.2.4.2. Treatment-related adverse effects

All 15 patients treated in the Lausanne cohort are alive with no extraocular tumor spread or metastasis at a mean follow-up of 18 months (range 3-84 months) after a mean of 6.5 injections (range 3-14). Most frequently observed ocular side effects were cataract in 6 eyes (40%), among which 5 eyes had received concomitant brachytherapy for a retinal or ciliary body relapses, and hypochromic heterochromia in 3 (20%). Noteworthy, no potential cytotoxic effect was detected in the three patients old enough to undergo endothelial cell count (personal observation). The technique of intracameral injection having been only recently developed further studies with more cases and longer follow-up will be needed to conclude on long-term adverse effects.

7.2.4.3. Potential alternatives to intracameral chemotherapy for aqueous seeding

Aqueous seeding control was also reported in isolated or limited case series by means of intravenous chemotherapy combined with intravitreal and periocular topotecan (Rao et al., 2018a) or brachytherapy (Shields et al., 2016c). In the case from India, it is not possible to determine how tumoricidal concentration was achieved to control aqueous disease and which of the 3 routes of delivery and 4 injected drugs or combinations of drugs contributed to the result (see Fig 20). In the series from Philadelphia, 3 eyes with anterior diffuse retinoblastoma devoid

of any retinal or vitreous involvement, achieved complete remission at a mean follow-up of 35 months by means of iodine plaque therapy delivered to the entire anterior segment. All eyes needed cataract surgery within a mean interval of 16 months. With such an approach, other delayed, potentially sight-threatening complications are to be feared, such as radio-induced damage to the trabeculum and/or corneal limbal stem cells.

7.2.5. *Periocular chemotherapy*

Periocular chemotherapy with carboplatin or topotecan was introduced in the 1990s, generally as an adjunct to systemic chemotherapy or cryotherapy in order to increase the intraocular drug concentration (see 7.1.5). The periocular delivery can be obtained using subconjunctival (Abramson et al., 1999b) or sub-Tenonian (Francis et al., 2015) injections with or without echographic localization (Cebulla et al., 2009). Different drug vehicles have been tested, including fibrin sealant (Mallipatna et al., 2011) or nanoparticles (Kalita et al., 2014).

In a long-term study on periocular carboplatin (20mg in 2ml) (Marr et al., 2012), only 2/13 eyes (15%) showed long-term complete response to periocular carboplatin alone, indicating inefficiency if used as monotherapy. Reported adverse local effects could also be quite severe, including ocular motility restriction (Mulvihill et al., 2003), orbital fat atrophy and fibrosis (Kim et al., 2010b) as well as optic nerve atrophy and subsequent blindness (Schmack et al., 2006), relegating periocular carboplatin to a marginal role, if any, in the conservative management of retinoblastoma.

A phase I study on periocular topotecan showed that 2 mg of the drug diluted in 2 ml normal saline could be administered without significant toxicity (Chantada et al., 2009). A retrospective study on periocular topotecan at a median dose of 0.18 mg/kg (range 0.5-2.8 mg) in 10 eyes, reported efficacy restricted to tumor reduction for group A and B eyes, which still required,

however, complementary focal treatments to be controlled. Periocular topotecan combined with systemic chemotherapy was also found to be significantly inferior to intra-arterial melphalan for salvage treatment of relapsed retinoblastoma (Schaiquevich et al., 2013).

Since the availability of intra-arterial and intravitreal chemotherapy, indications for periocular chemotherapy are limited to consolidation therapy of recurrent resistant tumors at the posterior pole in combination with other focal therapies. The recent description of long-acting episcleral chemotherapy implant (chemo-plaque) could revive the periocular route of drug delivery in advanced retinoblastoma (Carcaboso et al., 2010; Pontes de Carvalho et al., 2006). Two prospective studies are currently open in the United States using a device for sustained controlled-release of topotecan for primary unilateral group D and for eyes that have failed standard available therapy (<http://grantome.com/grant/NIH/RC3-CA150730-01>).

7.2.6. Focal treatments modalities

Focal treatments, including cryotherapy, hyperthermia, photocoagulation and brachytherapy, are aimed at destroying small intraocular tumors confined to the retina without the need of treating the whole eye. Because of their efficacy with few adverse effects, they represent important modalities in the therapeutic arsenal against retinoblastoma. Current indications of focal therapies include first line use in group A, macula/or papilla-sparing group B tumors, second line or consolidation after chemoreduction/chemothermotherapy (see 7.2.1.3.1), and salvage treatment of accessible recurrent retinal tumors. There is presently no consensus regarding the need of consolidation focal treatments following intra-arterial chemotherapy and their potential role in preventing recurrence as observed post chemoreduction (Table 11). Finally, although successful treatment of localized intravitreal seeding has been occasionally reported with brachytherapy (Abouzeid et al., 2008; Francis et al., 2013b) or cryotherapy (Abramson and

Scheffler, 2004a), these treatment modalities are not, however, primarily aimed at the control of vitreous disease.

All focal treatments have in common to be performed under general anesthesia. The choice of the treatment modality depends mainly on the tumor size and location. The number of sessions is adapted to the tumor response to treatment, with regression to a flat scar or totally calcified tumor being the ideal endpoint of treatment. If needed, they can be repeated (usually every 2-4 weeks) or used in combination. Presence of fish-flesh tissue is associated with a higher recurrence rate compared to calcified tissue, but corresponds to a complete response if stable over the follow-up (Ghassemi et al., 2013; Schueler et al., 2006)

7.2.6.1. Cryotherapy

Cryotherapy destroys small tumors (usually up to 2 mm high and less than 3 mm diameter) by freezing them at -80°C , resulting in intracellular ice formation and plasmic membrane rupture of tumor and endothelial cells, visualized by the presence of petechias at the time of recirculation. The treatment is performed with a cryoprobe directly applied to the outer surface adjacent to the tumor with a triple freeze-thaw technique allowing the iceball to completely cover the tumor. Cryotherapy is ideally used to treat pre-equatorial tumors, but lesions located more posteriorly may be reached by placing the cryoprobe in the sub-Tenon space after opening the conjunctival cul-de-sac. The treatment leaves a chorioretinal scar and has been reported to eradicate up to 90% of the treated lesions (Abramson and Scheffler, 2004b). The most frequently observed complication is transient conjunctival chemosis, whereas retinal tears and detachments, vitreous hemorrhage and transient subretinal fluid have been infrequently reported (Anagnoste et al., 2000; Hamel et al., 2000; Shields et al., 1993b).

7.2.6.2. Hyperthermia

Hyperthermia induces a cytotoxic effect on the tumors by heating them up to sub-coagulation temperatures of 45°C to 60°C. It is applied using an 810-nm infrared diode laser applied transpupillary on a continuous mode, either by indirect ophthalmoscopy and a 20 Diopter magnifying lens or with an adaptor coupled to an operating microscope through a three-mirror lens contact. Compared to the indirect ophthalmoscopy where the spot size is variable, the microscope adaptor allows to set the spot size between 0.3 and 2.0 mm as well as to achieve uniform delivery of the laser on the lesion. In the absence of temperature monitoring during treatment, the laser power setting is entirely empirical and varies from 100 to 1200 mW adapted to the tumor size, location and underlying level of pigmentation. The duration of treatment depends on the observed clinical response (good signs being whitening of the treated lesion and micro-hemorrhage on its surface) and usually varies from 5-30 minutes. Small naïve tumors usually respond quickly, whereas white avascular or calcified tumors show no changes due to limited energy transfer. The problems regarding the setting of energy and duration could be overcome in the future by the implementation of real-time temperature determination using opto-acoustic technology (Brinkmann et al., 2012). Ideal for posterior pole tumors, hyperthermia can also be used for peripheral tumors after appropriate indentation of the lesion.

Hyperthermia resolves over 90% of small tumors (≤ 1.5 mm in base diameter) without the need of any other treatment (Abramson and Scheffler, 2004a). Indocyanine green (Grobner et al.), which has an absorption peak similar to the emission of the diode laser and thought to promote a potentiated laser response in ICG retaining tissue, has been reported to be successful for tumors refractory to thermotherapy after intravenous or intra-arterial chemotherapy (so called ICG-enhanced thermotherapy) (Francis et al., 2013a). Hyperthermia can also be delivered 1-2 hours

after intravenous carboplatin, so-called chemothermotherapy, to potentiate its cytotoxic effect by virtue of a synergetic effect between heat and drug at the time of its plasmatic peak (Murphree et al., 1996; Lumbroso et al., 2002). With the advent of intra-arterial chemotherapy, chemothermotherapy is less frequently used.

Complications of transpupillary thermotherapy include iris atrophy with or without concomitant focal lens opacities, tumor seeding by rupture of the internal limiting membrane, retinal fibrosis, rhegmatogenous retinal detachment and vascular occlusion (Gombos et al., 2006; Shields et al., 1999; Tawansy et al., 2006).

7.2.6.3. Photocoagulation

Photocoagulation can be done with a green (532-nm) laser, continuous wave Nd:YAG (1064-nm) or infra-red (810 nm) transpupillary laser (Hamel et al., 2000) and enables tumor destruction indirectly by occluding tumor vessels (532-nm) or directly by generating heat with protein-denaturing temperature in excess of 65°C (coagulation) within the treatment spot. Complications include retinal fibrosis, traction, and vitreous seeding secondary to rupture of the internal limiting membrane (Gombos et al., 2006).

Transscleral diode laser (810 nm) can be used to destroy small peripheral tumors with a power setting varying between 1000 and 2000 mW and duration of 1-2 sec, applied until a whitening of the tumor is observed by indirect ophthalmoscopy. The laser probe has a focus of 1 to 2 mm of penetration into the eye and is thus only suitable to treat small peripheral tumors up to one disc diameter, but with the advantage to induce a much smaller chorioretinal scar than cryotherapy.

7.2.6.4. Brachytherapy

Brachytherapy is used for solitary, medium-sized tumors (up to 10 mm in diameter and 6 mm in height) and located more than 3 mm from the optic disc or fovea. Most commonly employed

isotopes are iodine 125 (I^{125}) or ruthenium 106 (Ru^{106}) and calculations are made for a delivered dose of 40 Gy to the apex of the tumor. Both types of plaque allow a similar tumor control rate of up to 95% of the treated cases at a 5-year follow-up (Schueler et al., 2006; Shields et al., 2001). However, radiation-related complications, including proliferative and non-proliferative retinopathy, radiation-induced maculopathy/papillopathy, subcapsular cataract and neovascular glaucoma are less frequent with the beta-emitting Ru^{106} because of its limited axial and lateral dosimetry compared to the gamma-emitting I^{125} (Fluhs et al., 2004).

7.3. Disease and treatment-related complications

The conservative treatment of retinoblastoma can be complicated by various conditions, such as cataract, rhegmatogenous/tractional retinal detachment or secondary neovascularization which, left untreated, may compromise the active tumor-related management or the vision/globe preservation of eyes considered in remission. In the era of intra-arterial and intravitreal chemotherapy, such complications are actually representing up to 50% of the indications to secondary enucleation (Eagle et al., 2011; Pavlidou et al., 2015) compared to less than 5% in the era of systemic chemotherapy (Balaguer et al., 2009; Chantada et al., 2007), and this rate is expected to increase in the future as more eyes with very advanced disease are now offered salvage treatments.

This section deals with the most frequently encountered complications during conservative management of retinoblastoma. Whereas some of them (amblyopia, cataract, retinal detachment and secondary neovascularization) can be offered specific treatments, others, related to drug toxicity and/or drug delivery procedure (chorioretinal complications after intravitreal or intra-arterial chemotherapy) are irreversible, and efforts should be made to prevent them until less toxic therapies become available.

Among the treatable conditions, early amblyopia management is without doubt one of the most important active measure to achieve vision preservation. As there is now sufficient evidence that useful vision can be obtained in the majority of affected eyes, including those with macular involvement, each time a visual potential is not given a chance to be realized can be considered a partial treatment failure (see 7.3.1).

Other conditions, such as cataract (see 7.3.2) or retinal detachment (see 7.3.3) may require invasive procedures, considered hazardous in the context of retinoblastoma. However, the introduction of safety-enhanced interventions can overcome the fear of potential tumor spread and should enable intraocular surgery to be considered in selected cases. As a rule, the decision to proceed with surgery should be based on the status of the contralateral eye, the estimated visual potential expected to be achieved after the intervention and, of course, full parental informed consent on the risk and benefits of the procedure.

7.3.1. Amblyopia, strabismus and nystagmus

The raising concern in preservation of visual function while managing ocular salvage has become noticeable in the increasing number of recent publications in where visual acuity is considered as one of the success criteria of surviving eyes after treatment (Chan et al., 2009; Demirci et al., 2005; Hall et al., 1999; Kim et al., 2010a; Manjandavida et al., 2014; Narang et al., 2012; Scheffler et al., 2007; Suzuki et al., 2011; Tsimpida et al., 2013; Weiss et al., 1994). Few authors, however, have addressed the importance of active visual reeducation in retinoblastoma eyes (Greenwald and Strauss, 1996; Watts et al., 2002). At Jules-Gonin Eye Hospital, a retrospective study was conducted to compare the visual outcome in 3 groups of children with a minimum of 4 years of age at last visit, all having fovea-involving tumors. The first group (n=32) included patients with bilateral disease having undergone enucleation of one

eye, these considered to be undergoing permanent occlusion. The second group (n=27) included only unilateral retinoblastoma patients undergoing active patching of the unaffected eye. The third group (n=13) included patients with unilateral retinoblastoma that failed to comply with amblyopia treatment. The median best corrected visual acuity was 0.7logMAR, 1.1logMAR and 1.7logMAR for groups 1, 2 and 3 respectively. There was a highly significant difference of visual acuity between the monophthalmic group 1 and the treated group 2 ($p=0.001$), and also between group 2 and the untreated group 3 ($p=0.016$), demonstrating the benefit of active visual reeducation in all cases with foveal involvement. In addition, the significant statistical difference between groups 1 and 2 suggests that a more aggressive amblyopia treatment regimen could even increase the visual gain (Fig 24).

The visual deprivation resulting from disease or treatment-related foveal damage or obscuration following hemorrhage, seeding or cataract, can cause not only amblyopia but also strabismus and possibly nystagmus, these in turn having a potential negative impact on quality of life. In a report on the visual outcome in patients with macular involvement treated by chemoreduction and focal therapy, strabismus, mostly exotropia, was noted in 16% of the patients (Scheffler et al., 2007). More recently, in a study on the long-term functional results in patients with group D eyes, 22% had nystagmus, and 60% of bilaterally salvaged patients had strabismus with exotropia being the most common type (Fabian et al., 2017a). By univariate analysis, strabismus and foveal tumor at presentation were found to be significantly associated with strabismus at final follow-up ($p=0.043$), while only foveal involvement was found to be significant ($p<0.001$) on multivariate analysis (Fabian et al., 2018). It should be stressed that conservation of a misaligned eye can cause as much embarrassment as an artificial eye, especially in teenagers. Strabismus surgery can be envisaged once growth is completed.

Similarly, an abnormal head posture due to nystagmus can also benefit from muscle translocation in order to redress the neutralized zone as close as possible to the primary position. Both procedures have been successfully performed in Lausanne (unpublished data).

7.3.2. Cataract

7.3.2.1. Incidence

During the irradiation era, cataract was a frequent treatment-induced complication, developing usually within two years following the treatment in up to 87% of the eyes treated with external beam irradiation (Blach et al., 1996; Fontanesi et al., 1996) and up to 37% of those treated with lens sparing technique or stereotactic conformal radiotherapy (Pica et al., 2011; Sahgal et al., 2006; Scott et al., 1999). Since retinoblastoma is now managed without external irradiation, cataract has become a less frequently encountered problem, but can still complicate brachytherapy (Abouzeid et al., 2008; Murakami et al., 2012; Schueler et al., 2006; Shields et al., 2006b), transpupillary thermotherapy (Shields et al., 1996), intravitreal (Francis et al., 2014; Ji et al., 2016; Munier et al., 2012a; Shields et al., 2016b) and/or intracameral chemotherapy (Munier et al., 2018). Radiation exposure during intra-arterial chemotherapy (from 5.55 to 191 mGy per eye) is below the reported doses for cataract formation (500mGy) (Thampi et al., 2013).

7.3.2.2. Cataract surgery

The risk of tumor spread in eyes with active retinoblastoma undergoing cataract surgery has since long been recognized (Brooks et al., 1990; Honavar et al., 2001) and requires specific security guidelines. A minimum of 6 to 12 months disease-free interval before surgery is recommended (Brooks et al., 1990; Honavar et al., 2001; Miller et al., 2005; Osman et al., 2011), whereas eyes where fundus view is lost sooner are usually treated with enucleation (Berry et al.,

2018a; Brennan et al., 2015). Providing that specific additional precautions are followed, however, such eyes, in selected cases, can still be managed conservatively.

General recommendations include performing clear cornea small incisions rather than pars plana or scleral tunnel to avoid any sub-Tenonian or sub-conjunctival contamination. The incisions should be secured with sutures at the end of the intervention and intraocular fluids sent for cytopathologic analysis to exclude retinoblastoma cells. Posterior capsulorhexis associated with anterior vitrectomy remains subject of controversy, since it can cause iatrogenic aqueous contamination (Munier et al., 2018). The dilemma of whether to leave the posterior capsule intact or not is particularly crucial in children younger than 5 years of age in whom it is difficult to perform YAG laser capsulotomy, but who at the same time are more likely to present a shorter time interval for treatment-free follow-up and relapses.

Specific recommendations prior surgery for eyes with a disease-free interval of less than 6 months should include an MRI to exclude extraocular disease extension, as well as two intravitreal chemotherapy injections one week apart in order to optimize safety prior the surgery. Cytology-negative results of the paracentesis performed at the time of the intravitreal injection should be confirmed before proceeding with the cataract surgery. Finally, in case of suspicion of active disease, posterior capsulotomy should only be done if the whole procedure is performed under infusion of melphalan at the concentration of 5 µg/ml. Following the above-mentioned recommendations, cataract surgery has been performed in 24 retinoblastoma eyes at Jules-Gonin Eye Hospital over the last 10 years, among which 9 had a disease-free follow-up of less than 6 months. No metastasis nor deaths were observed at a mean follow-up of 4.9 years (range 0.8-8.1 years) (unpublished data).

7.3.3. Rhegmatogenous retinal detachment

Retinal detachment, constitutive of exophytic growing tumor(s), is a frequently observed feature of retinoblastoma at presentation. Such detachments are purely exudative in nature and in the majority of cases complete resolution is achieved, regardless of the first line therapy administered (Berry et al., 2016b; Rowlands et al., 2018; Shields et al., 2012; Shields et al., 1997). Rhegmatogenous retinal detachments, however, can appear as a treatment-related complication and require appropriate management to allow further tumor control and/or eye preservation.

7.3.3.1. Incidence

Little is known about the rate of rhegmatogenous retinal detachment occurring during the conservative treatment of retinoblastoma. It was found to complicate 6% (5/83) to 11% (5/45) of the eyes treated conservatively during the external beam irradiation era (Bovey et al., 1999; Lim and Robertson, 2000). All those eyes except one treated with plaque brachytherapy only had received EBRT (n=9), with additional treatment including focal treatment (n=7) and intravenous chemotherapy (n=3). In the only report detailing the treatment of the eyes, those treated with external beam radiotherapy alone (0/16, 0%) or focal treatments alone (0/21, 0%) did not develop a retinal detachment (Bovey et al., 1999).

According to the single study focusing on vitreoretinal complications after systemic chemotherapy (Tawansy et al., 2006), the incidence of rhegmatogenous and/or tractional retinal detachment was limited to 1.2% (n=5/404) and the role of concomitant focal therapy in the context of chemotherapy-related impaired wound healing pointed out (Anagnoste et al., 2000; Tawansy et al., 2006).

More recently, in a series of 75 eyes (68 ICRB group D/E) treated with first line intra-arterial chemotherapy without concomitant focal treatments between 2008 and 2015 (Shields et al., 2017), 6 eyes (8%) developed rhegmatogenous retinal detachment. Extensive endophytic pattern was associated with a significantly higher risk for rhegmatogenous retinal detachment (5/22, 23%)($p=0.0073$), while only 4% (1/24) of the eyes with mixed tumor growth and none of 29 eyes with exophytic tumor growth developed a rhegmatogenous retinal detachment. Similarly, in a series of 74 eyes (69 IIRC group D/E) receiving first line intra-arterial chemotherapy without concomitant focal treatments between 2008 and 2018 in Lausanne (unpublished data), 12 cases (16%) of rhegmatogenous retinal detachment were observed. The incidence of the complication was also found to be significantly higher in endophytic eyes (9/32, 28%) ($p=0.015$), compared to eyes with mixed growth (3/24, 12%) or those with exophytic tumors (0/18, 0%). In both series, most cases (80%) of retinal detachment occurred within one month following the first intra-arterial chemotherapy, supporting the hypothesis that the complication develops from atrophic (often invisible) holes due to abrupt tearing forces caused by rapid necrotic tumor regression. Although specific data are missing, the lower frequency of rhegmatogenous retinal detachment observed after intravenous chemotherapy, suggests a more gradual tumor regression compared to intra-arterial chemotherapy. If true, advanced endophytic tumors may benefit of an initial course of systemic chemotherapy, as done in bridge therapy, before intra-arterial chemotherapy to initiate a less aggressive regression and prevent this complication (see 7.2.1.2.2).

Finally, rhegmatogenous retinal detachment, known to occur in around 0.01% of intravitreal injections in adult eyes (mostly myopic) (Meyer et al., 2011), was only reported following intravitreal chemotherapy in one single case (Smith et al., 2014; Suzuki et al., 2015).

7.3.3.2. Surgical repair

The violation of the state of metastatic grace by invasive surgical procedures, such as vitrectomy, in eyes previously treated for retinoblastoma (Honavar et al., 2001) or with unsuspected retinoblastoma (Kaliki et al., 2018; Shields et al., 2000; Spaulding and Fuhs, 1968) remains a major concern. With this in mind, most authors prefer minimally invasive methods for repair, including non-drainage scleral buckling with retinopexy (Bovey et al., 1999; Mullaney et al., 1997; Yousef et al., 2016) or with post-operative laser photocoagulation of a visible retinal break (Buerk et al., 2006). To avoid high intraocular pressure due to tightening of the band potentially resulting in central retinal artery occlusion, different strategies have been proposed, including intravenous mannitol infusion 1 hour before surgery, post-operative acetazolamide (Yousef et al., 2016), or per-operative anterior chamber paracentesis (Mullaney et al., 1997). Some authors recommend at least a 6- to 12-month disease-free interval before considering scleral buckling with external drainage or vitrectomy with cytopathologic evaluation of the intraocular fluids (Baumal et al., 1998; Lim and Robertson, 2000; Madreperla et al., 2000; Tawansy et al., 2006). In Lausanne, in the absence of signs of spontaneous retinal reapplication, an early *ab externo* approach with scleral buckling with no subretinal drainage is preferred, especially if the detachment occurs in a child's eye with visual potential, as a prolonged observation interval may compromise the functional outcome. An intra-operative anterior chamber paracentesis is performed systematically to allow sufficient tightening of the band. In case of inadvertent scleral perforation, aspiration and cytopathologic analysis of the fluid should be done and the operation field prophylactically immersed with 2 mg of topotecan (1 mg/ml). Following this strategy, complete retinal application was successfully achieved in 92% of the 12 (n=11/12) above-mentioned eyes (see 7.3.3.1) and partially but with an applicated fovea in 1 eye

at a mean follow-up of 4.4 years (range 0.5-9.0). All patients are alive with no metastases (unpublished data).

If an intraocular procedure is preferred, tumor reactivation should be ruled out as the cause of the detachment. Pars plana vitrectomy (23 or 25G) with melphalan irrigation (5 µg/ml) with or without silicone oil tamponade is possible (Yarovoy et al., 2015). In the case of retinal detachment occurring concomitantly to proliferative vitreoretinopathy, with or without retinal dehiscence, an intravitreal anti-VEGF injection can be successfully given one week prior to vitrectomy under melphalan infusion combined with retinotomy (Stathopoulos et al., 2018a).

7.3.4. *Secondary neovascularization*

Secondary neovascularization can develop during the conservative management of retinoblastoma and affect both posterior and anterior segments of the eye. Its occurrence has been associated with retinal ischemia secondary to chronic retinal detachment (Stathopoulos et al., 2018a; Tawansy et al., 2006), focal treatments (Ghassemi and Amoli, 2014; Kase et al., 2008; Scheffler et al., 2007), external irradiation (Archer et al., 1993; Hwang et al., 2017), plaque radiotherapy (Abouzeid et al., 2008; Albert, 1987; Francis et al., 2013b; Murakami et al., 2012), intra-arterial chemotherapy (Bianciotto et al., 2012; Stathopoulos et al., 2018a) as well as active tumor progression (Stathopoulos et al., 2018a) or recurrence (Kiratli et al., 2017; Stathopoulos et al., 2018a).

The exact incidence of secondary neovascularization prior the era of intra-arterial chemotherapy is unknown. In a retrospective study including all eyes treated with primary or salvage intra-arterial chemotherapy over a 10-year period in Lausanne, 20% (n=37/182) of the eyes, among which 30% (n=11/37) had active tumor, developed secondary neovascularization, at a mean interval of 9.6 months since the first injection and after a mean of 3 injections. In that

study, the incidence of secondary neovascularization was higher but not significantly after salvage compared to first-line intra-arterial chemotherapy (22%, 25/114 versus 18%, 12/68) (unpublished data), which is similar to the rates of 20% (17/85) after primary and 16% after salvage intra-arterial chemotherapy found by others over the same period of time (Ancona-Lezama et al., 2018). If heavily pre-treated eyes, particularly after external irradiation, are at higher risk to develop neovascular complications after intra-arterial chemotherapy remains to be established.

Neovascular complications in retinoblastoma, especially if concomitant with elevated intraocular pressure or intraocular hemorrhage, are usually followed by enucleation. Anecdotally, cases with no active tumor developing proliferative vitreoretinopathy associated with chronic retinal detachment have been treated with pars plana vitrectomy and membrane peeling (Tawansy et al., 2006; Warden and Mukai, 2006) and choroidoretinal neovascularization with Argon laser (Archer et al., 1993). The use of intravitreal anti-VEGF in the treatment of 35 eyes with secondary retinal and/or iris neovascularization was recently reported (Stathopoulos et al., 2018a). In this study, intravitreal anti-VEGF provided the conditions for further conservative management allowing an overall globe preservation rate of 51% (n=18/35) at a mean follow-up of 2.4 years. Eyes with no concomitant active tumor had a better globe salvage rate than eyes with active tumor (65% vs 25%) (Stathopoulos 2018a).

7.3.5. Chorioretinal complications

Chorioretinal complications are the most frequently reported intraocular complications of both intravitreal and intra-arterial chemotherapy, leading to the so-called intravitreal melphalan-induced chorioretinopathy and choroidal occlusive vasculopathy respectively. Both are

irreversible and can cause permanent visual loss if the macula is involved. Clinical features and risk factors of these adverse effects are discussed below. In addition, measures to reduce/prevent the intravitreal melphalan-induced retinal toxicity after intravitreal melphalan are proposed (see 7.3.5.1.3). Finally, a classification system to grade the as yet poorly described choroidal occlusive vasculopathy according to its extent is presented, in order to facilitate comparisons between studies reporting adverse effects and functional outcomes after intra-arterial chemotherapy in the future (see 7.3.5.2.1).

7.3.5.1. Intravitreal melphalan-induced chorioretinopathy

7.3.5.1.1. Clinical presentation, classification system and incidence

Intravitreal melphalan-induced retinopathy, also referred to as salt and pepper retinopathy, presents as an abrupt, irreversible, chorioretinopathy usually confined to the meridian of the needle entry site. The first visible signs are vascular in nature, resulting in an area of intra-retinal hemorrhages occurring within 2 days of the injection and evolving within one month to a well demarcated retinal atrophy with pigmented changes, associated with choriocapillary atrophy of various extents appearing progressively. A classification system of the intravitreal melphalan-induced chorioretinopathy was proposed to grade the retinal toxicity according to the location and extent of the retinal damage (Munier, 2014), with grade 1-3 referring to a pre- and retro-equatorial involvement sparing the macula, and grade 4 and 5 to a maculopathy and optic neuropathy respectively (Fig 25).

Overall, the melphalan-induced chorioretinopathy has been variously reported to occur in 0.8-43% of the cases (Smith et al., 2014). In a retrospective review of all eyes (n=90) receiving a first intravitreal treatment with melphalan at Jules-Gonin Eye Hospital over the last 10 years, 41% developed the complication, which was classified as grade 1 or 2 in over 90% of the cases.

Complications grade 4 and 5 were never observed in the Lausanne cohort (unpublished data) (Table 14).

7.3.5.1.2. Risk factors associated with intravitreal melphalan-induced chorioretinopathy

There are several potential risk factors for retinopathy following intravitreal melphalan, namely vitreous drug concentration, eye pigmentation and localization of drug delivery. These aspects are discussed in detail below.

7.3.5.1.2.1. Intravitreal concentration: the influence of the injected dose and vitreous volume considering age-related and tumor-related factors

The intraocular toxicity of intravitreal melphalan was initially investigated in preclinical studies on the rabbit showing that a vitreous concentration of 5.9 µg/mL, corresponding to a human injected dose of 24 µg, was not causing any electroretinographic (ERG) perturbation (Ueda et al., 1995). This was further confirmed in a study describing the innocuity of melphalan at a vitreous concentration of 5 µg/mL, but a 52% decrease of both a- and b-wave amplitudes at a concentration of 10 µg/mL, with necrosis of the inner nuclear layer and thinning of the outer nuclear layer (Shimoda et al., 2008). In humans, an immediate and permanent 5.8 mV decrease in ERG amplitude per injection of 30 µg was reported (Francis et al., 2014).

According to the above, it became accepted that it was efficient to inject a dose of 20-30 µg melphalan (Munier et al., 2012a). As previously discussed, intravitreal injection of 8 µg melphalan has been reported to be ineffective, whereas 50 µg has been associated with phthisis bulbi (Ghassemi and Shields, 2012). Potential risk factors for the severity of the melphalan-induced chorioretinopathy (grade 3 versus grade 1 and 2) were studied in a series of 90 eyes treated for the first time with intravitreal melphalan in Lausanne (see 7.3.5.1.1), including time to

develop the complication since the first injection, total intravitreal injected dose, and mean intravitreal drug concentration reached until the occurrence of the complication. Only intravitreal drug concentration integrating both age-related vitreous volume (Fledelius and Christensen, 1996; Teichmann, 2002) (Fig 26) and tumor volume (effective intravitreal drug concentration) was found to be significantly correlated with the severity of the melphalan-induced chorioretinopathy ($p=0.04$) (Table 14). To determine the effective intravitreal melphalan concentration, the formula below was used, in which the vitreous was estimated to occupy 80% of the eye volume and the tumors to be ellipsoids:

$$\text{Effective intravitreal drug concentration} = \frac{\text{Dose}}{(0.18 * e^{0.15*AL}) * 0.8 - \sum_{n=1}^N \frac{4}{3} * \pi * h_n * r_{1n} * r_{2n}}$$

For this calculation, the axial lengths (AL, in mm) were estimated according to age or the corrected age in case of prematurity. Height (h), longitudinal (r1) and transverse (r2) radii (in millimeters) of each tumor (n) were measured with ultrasonography (12 MHz). The volume occupied by each tumor was summed up and subtracted from the estimated vitreous volume.

These observations imply that tumor volume and not only age-matched vitreous volume is an important factor to consider when choosing the dose to be injected. Recommended melphalan doses for intravitreal chemotherapy according to age and percentage of the vitreous volume occupied by the tumor(s) are presented in Table 15.

7.3.5.1.2.2. Para-vitreous injections

Para-vitreous injections can lead to a toxic exposition of the retina, despite adequate intraocular melphalan dose. Thus, in eyes with an extensive posterior hyaloid detachment, injection into the

retro-hyaloid space may induce a grade 4 or 5 retinopathy (Aziz et al., 2017) (Fig 25). Similarly, injection into an open Cloquet's canal with subsequent filling of the virtual bursa premacularis may induce a grade 5 retinopathy (Fig 25).

To avoid paravitreal injections, ultrasonography should always be performed prior to the procedure, to inject preferentially in a tumor free quadrant where the posterior hyaloid is not detached. On the other hand, the correct positioning of the needle centrally behind the lens should be done under the operating microscope view, which also allows the visualization of a retro-lental bubble after initial injection of a small volume, indicating a smooth diffusion of the drug into the vitreous cavity. If no bubble can be seen, mis-injection into Cloquet's canal should be feared, requiring a reposition of the needle elsewhere where a bubble can be observed.

7.3.5.1.2.3. Intraocular pigmentation

Since melphalan has a tropism for the melanin of the retinal pigment epithelium (Süsskind et al., 2016), it would be reasonable to expect that more pigmented eyes may be at greater risk of having the complication. Some authors showed a significant reduction in ERG findings in eyes with brown iris and pigmented fundus compared to those with blue iris and blond fundus (Francis et al., 2017b). In the retrospective analysis of the above-mentioned cohort of patients treated in Lausanne (see 7.3.5.1.1) considering the presence or absence of the toxic retinopathy according to the iris color, the opposite was, however, observed, with patients having blue or green irides (n=28) presenting significantly more often the retinal complication than patients with brown irides (n=61) (16/28 57% vs 21/61 34%, p=0.04). In the eyes presenting intravitreal injection-induced chorioretinopathy, the total dose up to the complication was similar between the light- and dark iris groups (57 µg vs 53 µg), and average effective intravitreal drug concentration was within safety limits in both groups (7.9 µg/ml vs 6.6 µg/ml).

7.3.5.1.2.4. Concomitant drug interaction

It has also been suggested that concomitant intravitreal and intra-arterial melphalan may increase the number of adverse effects (Francis et al., 2017b). In the Lausanne cohort however (unpublished data), there were fewer cases developing melphalan-induced chorioretinopathy in the eyes which received intra-arterial melphalan two days apart from the intravitreal injection compared to those who were treated with intravitreal melphalan alone (19/52 (37%) vs 18/38 (47%), $p=0.39$, Fisher test)). Moreover, the eyes receiving combined treatment had also fewer cases with grade 2 and 3 intravitreal melphalan-induced chorioretinopathy (10/19 (52%) vs (12/18) 67%, $p=0.51$, Fisher test) and a longer time to retinopathy development (3.1 vs 2.1 weeks, $p=0.08$, Mann-Whitney U -test).

7.3.5.2. Choroidal occlusive vasculopathy

The occurrence of intraocular adverse effects after intra-arterial chemotherapy was first reported in 2011, (Munier et al., 2011), with choroidal occlusive vasculopathy being the most frequently observed (Bianciotto et al., 2012; Bracco et al., 2013; Eagle et al., 2011; Muen et al., 2012; Munier et al., 2011).

7.3.5.2.1. Clinical presentation, incidence and grading system

Clinically, choroidal occlusive vasculopathy, also referred to as choroidal atrophy, choroidal ischemia or choroidal infarction, is an irreversible adverse effect of intra-arterial chemotherapy first recognizable by sectorial or diffuse retinal pigment epithelial alterations evolving towards partial or complete choriocapillary and retinal pigment epithelial atrophy. Like intravitreal melphalan-induced chorioretinopathy, the severity of the choroidal vasculopathy can be graded according to its extent and impact on the visual outcome. A classification based on clinical

observations is proposed in Table 16 in the hope that it will help assessing the frequency and severity of this complication across groups (Fig 27).

Its incidence varies substantially in the literature ranging from 7% (Dalvin 2018) (Bianciotto et al., 2012) to 47% (Muen et al., 2012). This variability may be explained at least in part by a learning curve (Dalvin et al., 2018; Reddy et al., 2017), and/or by the fact that not all grades of the complication are necessarily reported (see Table 16). According to the Lausanne data collected over the last 10 years, this complication was observed in 21 of 560 injection procedures (3.8%) administered to 213 eyes (9.8%), and typically occurred after a mean number of 2.4 injections (range 1-5), distributed in 10% grade 1, 52% grade 3, 10% grade 4, and 28% grade 5.

7.2.5.2.2. *Factors associated with choroidal occlusive vasculopathy*

Different hypotheses have been proposed to account for the occurrence of choroidal occlusive vasculopathy, including the injection mode and technique of cannulation of the ophthalmic artery, the injected drug toxicity as well as intrinsic predisposing factors. In the literature, there is a decline in the reported vascular events over time, most likely due to a learning curve enabling complications of ophthalmic artery catheterization to be avoided, with the complication occurring in about 7-10% of the injected eyes (Dalvin et al., 2018; FLM unpublished data). However, when isolated choroidal atrophy (i.e. not associated with ophthalmic artery occlusion) and choroidal atrophy resulting from ophthalmic artery occlusion are cumulated, the overall choroidal vasculopathy incidence is between 10 and 15% of injected eyes (Ancona-Lezama et al., 2018; Dalvin et al., 2018).

Intra-arterial chemotherapy with melphalan or carboplatin has been shown *in vivo* to trigger vascular toxicity through endothelial cell inflammation and leukostasis, in a study without sham injection of saline to test the effect of the ophthalmic catheterization *per se* (Steinle et al., 2012).

This was later tested in another animal model, demonstrating no vascular damage following saline injection (Daniels et al., 2018). The role of the micro-catheter positioning at the ostium versus occlusive engagement into the ophthalmic artery, as well as the continuous versus pulsatile injection mode, have also been debated. The issue of occlusive vs non-occlusive technique of injection appears not to be primarily responsible for the choroidal occlusive complications, at least in the rabbit model (Daniels et al., 2019).

A dose-related ocular toxicity of melphalan was hypothesized by the London group (Reddy et al., 2017), who reported that visual complications may be minimized with age-adjusted melphalan dosages. However, such a dosage effect, at least within the published dose range, could not be demonstrated in a subsequent study from Philadelphia (Dalvin et al., 2018).

The relative insolubility of melphalan is another potential source of toxicity linked to the embolization of micro-crystals up to 20 μm in diameter if not vigorously shaken (Tse et al., 2013). Filtration of melphalan after reconstitution using a 0.22 μm filter has been advised but its use is not widely reported in the intra-arterial chemotherapy literature.

Finally, genetic predispositions to thromboembolism have been reported in association with choroidal occlusive vasculopathy, including sickle cell trait, prothrombin mutation, plasminogen activator inhibitor-1 polymorphism (Abramson et al., 2012b; Francis et al., 2012), as well as methylenetetrahydrofolate reductase (MTHFR) polymorphic alleles (Bohm et al., 2018). In the latter case, 4 patients were recently reported who inhaled nitrous oxide pre-intra-arterial chemotherapy resulting in an increase of homocysteine in the blood (Bohm et al., 2018). The exact role of these inherited thrombophilic traits among the patients with choroidal occlusive vasculopathy is still unknown.

7.4. Evaluation of quality of life

This review is sub-titled: “Alive, with good vision and no co-morbidity”. Even fully achieved, this medical goal needs to be confronted with patients’ everyday life. How survivors themselves, as well as those around them, cope with the disease seems especially important (Hamama-Raz et al., 2012; van Dijk et al., 2009). To date, however, this issue has been only poorly addressed (Moll et al., 2013). Even if most of long-term retinoblastoma survivors and their parents report an overall good quality of life and limited psychosocial problems in comparison to peers (van Dijk et al., 2007a; van Dijk et al., 2007b; Weintraub et al., 2011; Sheppard et al., 2005), confrontation with the consequences of the disease still last for their entire lives. The perception of the disease as well as the psychosocial functioning and development of an affected child is influenced by the parents’ ability to cope with the stress associated with diagnosis and treatment (Hamama-Raz et al., 2012; van Dijk et al., 2007a; van Dijk et al., 2007b; Weintraub et al., 2011). In addition, patients not only have to withstand a delay in visuo-motor integration in comparison with their healthy peers (Ross et al., 2001), but also a life-long fear of developing recurrences, second primary malignancies, further loss of vision and passing on the disease to their offspring (van Dijk et al., 2010). When parents had to compare the quality of life of their affected children with healthy siblings, their report was significantly worse regarding the former (Batra et al., 2016a). A study comparing self (child)- versus parental evaluation of health-related quality of life showed that parents reported worse on emotional domains (Batra et al., 2016b). Over the last few decades, a substantial number of patients, mostly those having received irradiation or experienced restrictions in daily life and bullying at school, needed special educational services and psychological guidance or treatment (Sheppard et al., 2005; van Dijk et al., 2010). Finally,

fewer marriages and more divorces have been reported in retinoblastoma survivors treated by enucleation and/or external beam radiotherapy (Byrne et al., 1995).

A multi-disciplinary retinoblastoma team should ideally include a dedicated and fully integrated psychological and spiritual accompanying person. Such a presence benefits all families, regardless of religious confession, from diagnosis to final follow-up and, most importantly, during the delicate therapeutic phases, not only to facilitate communication between the patients' family and the medical and nursing staff, but also to provide support for both parents and staff when confronted with enucleation, relapse or even death. The quality of attention paid to emotional, psychological and spiritual aspects of patient care has proved to be of inestimable importance to the quality of life (Lima et al., 2013).

8. Future directions and conclusion

Historically, therapeutic innovation in the conservative management of retinoblastoma was driven independently of randomized clinical trials, often in the absence of preclinical studies, mostly due to the orphan nature of the disease. Over the last decade, virtually all steps forward were taken by challenging orthodoxy with new administration routes of known drugs subsequently evaluated in non-randomized retrospective studies. In the future, therapeutic breakthroughs should benefit from an increasing contribution of translational research and initiatives supporting rare disease consortium such as the European Retinoblastoma Group (EuRbG), favoring prospective multicentric clinical trials. In addition, the availability of new animal models for preclinical studies is expected to potentially improve treatment options (see 8.1). On the other hand, the advent of “omics” technology (Golabchi et al., 2018; Hudson et al., 2018; Kapatai et al., 2013; Kooi et al., 2016b; Naru et al., 2017) is expected to allow not only a better understanding of the mechanisms underlying tumor biology (*MYCN* vs non-*MYCN*) and

progression (somatic chromosomal copy-number alterations, epigenetic changes) including seeding, metastasis and relapse, but also novel diagnostic and therapeutic applications. Last but not least, this section also proposes to unveil some new potential therapeutic avenues, including gene therapy (see 8.2), cell-of-origin targeted therapy (see 8.3), personalized management (see 8.4) as well as new drugs (see 8.5) or new Galenic formulation of known drugs (see 8.6).

8.1. Animal models

There is a surprising paradoxical discrepancy between the evolutionary conserved RB-E2F pathway in most eukaryotes lineage species, including animals and plants (Cao et al., 2010) and the fact that *RB1* loss of function causes preferential retinal oncogenesis as a human-specific condition, with no proven equivalent in the animal kingdom other than anecdotal cases of sporadic unilateral retinoblastoma-like tumors (*RB1* status not investigated) in the dog and lama (Syed et al., 1997; Fugaro et al., 2005; Regan et al., 2013). This has imposed alternative models based in xenografts or genetically engineered mouse models (GEMMs) to conduct translational research in the field. Methods to monitor tumor growth and allow accurate evaluation of new treatments evaluation such as small animal imaging systems and diagnostic equipment adapted to the preclinical setting have been developed in parallel (Nemeth et al., 2011).

Intraocular xenografts using Y79 and WERI-Rb1 cell lines established more than 40 years ago from enucleated eyes of patients who received no previous treatment have been widely employed in immunodeficient mice where they can reproduce the inactivation of *RB1* and metastasize (Y79) to the central nervous system (CNS) (Chevez-Barrios et al., 2000; Lee et al., 1988; McFall et al., 1977; Reid et al., 1974). Over the last years, the orthotopic transplantation of fresh tumor samples or early stage primary cultures to mice has been prioritized, producing very relevant xenografts with striking anatomic and genetic resemblance to the patient disease

(Pascual-Pasto et al., 2016; Xu et al., 2009; Zhang et al., 2012). A few institutions have developed programs to systematize the sampling of viable tumor fragments or vitreous seeding from enucleated eyes at the pathology laboratory (Benavente and Dyer, 2015; Sastre et al., 2009). Serum-free, growth factor-supplemented culture medium is used to expand the small tumor fragments obtained from the enucleated eyes and maintain retinoblastoma cells in culture. This method selects stem-like cells that grow as floating tumor spheres resembling tumor seeding, as opposed to culture medium with bovine serum that promotes cell differentiation (Bond et al., 2013; Ma et al., 2011; Pascual-Pasto et al., 2016).

Alternatively, different genetically engineered mouse models (GEMMs) have been developed through manipulation of the genes in the Rb pathway (Benavente and Dyer, 2015). Inactivation of the *Rb* gene alone, however, is not sufficient to develop retinoblastoma in mice (Jacks et al., 1992), which need therefore concurrent inactivation of Rb-related genes. For instance, conditional deletion of *Rb* and *p130*, through the Pax6a promoter, induces bilateral intraocular tumors with 100% penetrance by one month postnatal, subsequent vitreous and aqueous seeding, as well as CNS and lymph node metastases (MacPherson et al., 2007). Other developed GEMMs include concurrent inactivation of *Rb* and *p107* (Chen et al., 2004; Robanus-Maandag et al., 1998), *Rb*, *p107* and *p130* (McEvoy et al., 2011), or *Rb* and *p107* combined with *Arf* deletion, or amplifications of *MDMX* and *MYCN* (Conkrite et al., 2012; McEvoy et al., 2011; Wu et al., 2017). Most of them, however, have the disadvantage of incomplete penetrance and/or long latency, making it difficult to interpret the efficacy of new therapies, or do not reproduce the patient outcome to treatments as compared to xenografts that replicate patient response closely (Brennan et al., 2011).

Finally, various tumor and non-tumor bearing animal models have been used to study the pharmacokinetic of different drugs depending on the intraocular delivery model. Large animals such as primates, pigs and rabbits are preferentially chosen because of their appropriate size, their similar proportion between ocular compartments compared to human eyes and the possibility they allow for repeated or continuous sampling of the ocular fluids and the cannulation of the ophthalmic arteries (Carcaboso et al., 2007; Carcaboso et al., 2010; Daniels et al., 2018; Ditta et al., 2012; Schaiquevich et al., 2012c; Vézina, 2013). Macaques and rabbits have been successfully used to characterize the toxicity of intra-ophthalmic artery and intravitreal melphalan in the retina (Francis et al., 2014; Shimoda et al., 2008; Wilson et al., 2011).

8.2. *Gene therapy*

The well-defined genetic origin of retinoblastoma and its confinement within a small, accessible and visible location nicely fit the conditions for a gene therapy approach. Transduced genes may generate therapeutic products to be released locally, or products that target dysfunctional oncogenic pathways within the tumor cells.

The first gene therapy to achieve a phase I clinical trial in retinoblastoma used a replication-deficient adenoviral vector (AdV-TK), carrying the herpes simplex thymidine kinase, a suicide gene activated by ganciclovir. The trial included eight retinoblastoma patients facing imminent enucleation due to active vitreous seeding (Chevez-Barrios et al., 2005; Hurwitz et al., 1999). Repeated intravitreal injections of AdV-TK and concomitant intravenous ganciclovir were proven feasible and safe. Treatment-related vitreal inflammation was controlled with corticosteroids. However, despite initial resolution of active vitreous seeds, all eyes were finally enucleated secondary to retinal tumor progression, likely because adenovirus 5 vectors have

limited ability to penetrate, infect and transduce retinal tumors, or alternatively because the expression of the genes transduced by adenoviruses is only short-term. Subsequent studies on this vector found that a modified adenovirus 5 fiber gene achieved better cell infection and protein expression (Mallam et al., 2004). Another gene therapy approach explored local transduction of *IFN1B*, encoding interferon- β , an antineoplastic molecule, with an adeno-associated virus, showing antitumor activity against Y79 xenografts *in vivo* (Shih et al., 2009).

Other viral vectors for retinoblastoma gene therapy induce an oncolytic effect, such as the adenoviruses H101 (Song et al., 2010) and Ad-TERT p-E1 (Wang et al., 2013), which have been tested in preclinical studies. Currently, the most advanced oncolytic adenovirus for retinoblastoma is VCN-01, designed to replicate selectively in tumor cells with high abundance of free E2F-1 transcription factor activity due to the dysfunctional RB1 pathway (Pascual-Pasto, 2018). The genome of VCN-01 contains one deletion in the E1A gene that precludes viral replication in RB1-functional cells and one insertion of an *E2F1* promoter under the *E1A* gene that favors replication in cells with free E2F-1 (Rodriguez-Garcia et al., 2015). In mice, the intravitreal administration of VCN-01 in orthotopic retinoblastoma xenografts infected and killed tumor cells, improved ocular survival significantly compared to standard of care chemotherapy, and reduced the frequency of CNS metastases (Pascual-Pasto, 2018). In immunocompetent rabbits, this treatment induced minor local inflammation and did not replicate in retinas (Pascual-Pasto, 2018). Intravitreal VCN-01 is currently undergoing a phase I clinical trial in children with refractory retinoblastoma (NCT03284268) and preliminary results of the 2 first patients treated have been published, showing the feasibility and safety of the procedure and evidence of viral replication markers in tumor cells (Pascual-Pasto, 2018).

8.3. Cell-of-origin targeted therapy

Two approaches that target intrinsic cell-of-origin features remain possible: First, if the cell-of-origin signaling circuitry that drives tumorigenesis represents an embryonic developmental stage, it may be possible to target this circuitry without affecting other cell types in retinoblastoma patients. For example, we know that the cone precursor proliferative response depends on intrinsically highly expressed proteins including RXR γ , TR β 2, MYCN, and MDM2 (Xu et al., 2014). Several preclinical agents are available to attack these troublemakers and could be considered for translational development. Second, it has been suggested that cancer pathways may be epigenetically deregulated as a direct or indirect result of *RBI* loss (Zhang et al., 2012). It might thus be useful to target the cone precursors' epigenetic responses to pRB loss.

8.4. Personalized therapy and liquid biopsy

Unprecedented opportunity for customized therapy is emerging by capitalization on individual liquid biopsy made possible since the introduction of the safety-enhanced intravitreal chemotherapy. Now that aqueous humor can be routinely and repeatedly sampled from one or both eyes in retinoblastoma patients as a non-invasive surrogate of tumor biopsy (Berry et al., 2017c), new insights into tumor biology can be undertaken based on all aqueous humor content, including cell-free DNA, exosomes and proteins.

The proof of principle of this approach has been recently established in a retrospective study, significantly correlating somatic chromosomal copy-number alterations (6p gain) in cell-free DNA (cf-DNA) from aqueous humor and secondary enucleation (Berry et al., 2018b). If clinically validated, this remarkable achievement may influence management, especially in terms of treatment monitoring and/or personalized therapies based on specific tumor profiles and to

distinguish MYCN from non-MYCN retinoblastomas. In addition, this technology is opening an avenue to study the chronology of mutations associated with tumor progression.

Another seminal paper (Gerrish et al., 2019) now further expands the applications of cf-DNA analysis in liquid biopsy of retinoblastoma patients by demonstrating that i) somatic *RBI* mutations can be successfully detected (even at concentrations < 0.1 ng/ μ l), including single-nucleotides and copy-number variants, ii) mutant *RBI* allele frequency in cf-DNA tightly reflects tumoral zygosity allowing the distinction of loss of heterozygosity (LOH) vs non-LOH events, iii) $>90\%$ of cf-DNA in the aqueous humor is of tumoral origin, and iv) aqueous cf-DNA content appears to be proportional to the tumor burden. This outstanding advance was obtained by sequential targeted capture enrichment for the promoter and exonic regions of the *RBI* gene, highly heterozygous single nucleotide polymorphisms and non-polymorphic regions across chromosome 13, followed by massively parallel sequencing. This breakthrough is expected to significantly impact the current clinical management of retinoblastoma, characterized by a dramatic reduction of enucleation rates even in advanced cases. In apparent non-hereditary retinoblastoma patients, eye preservation therapy is drying up the only existing source of tumor DNA for somatic mutation detection prior to genomic confirmation and thus restraining the identification of germline carriers (15%) in this population. Now that cf-DNA from the conserved eye is available for somatic mutational screening, accurate genetic counseling and clinical surveillance in the family at large can be re-enforced. Furthermore, the identification of compound heterozygote, homo- or hemizygote somatic hits in the aqueous humor of unilateral retinoblastoma, as well as one or more heterozygote somatic hits in germline patients with multifocal tumors, may provide biological markers for personalized detection of minimally disseminated disease in bone marrow biopsy and lumbar puncture.

8.5. *New drugs or combinations of drugs*

Among the increasing number of new potential targets against retinoblastoma, different therapeutic strategies can be envisaged targeting either the micro-environment (e.g. angiogenesis or glycolytic inhibitors) (Houston et al., 2013) or specific retinoblastoma pathways (e.g. inhibitors of SIK or MDM2/MDMX-p53 interaction) (Pritchard et al., 2016). Alternatively, new molecules potentiating the anti-tumor activity of known treatment modalities, including chemotherapy, radiotherapy or hyperthermia, may also lead to improved clinical outcome. For example, improvement of chemohyperthermia may result from combined administration of carboplatin and inhibitors of heat shock proteins, which are rapidly produced to buffer stress, including but not restricted to heat damage, and enable recovery in virtue of their anti-apoptotic properties. Interestingly, HSP70 and HSP90 have been identified in retinoblastoma cells (Jiang et al., 2008), justifying further investigations on the role of heat shock protein inhibitors as potentiating molecules of hyperthermia.

8.6. *New Galenic formulation of known drugs*

One of the limitations of chemohypethermia is that less than 1% of the intravenous carboplatin reaches the tumor, while the entire body is exposed to toxic adverse effects. An alternative approach using small chemotherapeutic molecules activated by heat only could therefore be beneficial by reducing the dose without impacting efficacy (Figure 28). Small-molecule thermoresponsive anticancer drugs that synergize with thermotherapy have been reported and validated in an adenocarcinoma-bearing murine model (Clavel et al., 2015).

8.7. Conclusion

Over the last decade, the advent of new techniques of chemotherapy delivery directly into the eye (namely intra-arterial, intravitreal and more recently intracameral injections) radically transformed the conservative treatment of retinoblastoma, allowing not only salvage of the majority of eyes, even those with advanced disease, but also eradicating external beam radiotherapy, as well as significantly reducing the rate of primary and secondary enucleations and the indications for systemic chemotherapy. These major advancements were obtained while maintaining a very low rate of metastatic disease, but our ability to detect minimally disseminated disease, as well as to treat CNS disease, still needs improvement to achieve zero-tolerance regarding metastatic death. Finally, in order to promote a better quality of life from diagnosis throughout the active therapeutic period and over the patients' whole lifetime, further efforts should aspire to shorten the time to success, contain adverse side effects, improve visual outcome, reduce the incidence of second primary neoplasms, and better support the needs of the survivors and their families.

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References

- Abouzeid, H., Balmer, A., Moulin, A.P., Mataftsi, A., Zografos, L., Munier, F.L., 2012. Phenotypic variability of retinocytomas: preregression and postregression growth patterns. *Br J Ophthalmol* 96, 884-889.
- Abouzeid, H., Moeckli, R., Gaillard, M.C., Beck-Popovic, M., Pica, A., Zografos, L., Balmer, A., Pampallona, S., Munier, F.L., 2008. (106)Ruthenium brachytherapy for retinoblastoma. *Int J Radiat Oncol Biol Phys* 71, 821-828.
- Abouzeid, H., Schorderet, D.F., Balmer, A., Munier, F.L., 2009. Germline mutations in retinoma patients: relevance to low-penetrance and low-expressivity molecular basis. *Mol Vis* 15, 771-777.
- Abramson, D.H., Beaverson, K., Sangani, P., Vora, R.A., Lee, T.C., Hochberg, H.M., Kirsztot, J., Ranjithan, M., 2003. Screening for retinoblastoma: presenting signs as prognosticators of patient and ocular survival. *Pediatrics* 112, 1248-1255.
- Abramson, D.H., Beaverson, K.L., Chang, S.T., Dunkel, I.J., McCormick, B., 2004. Outcome following initial external beam radiotherapy in patients with Reese-Ellsworth group Vb retinoblastoma. *Arch Ophthalmol* 122, 1316-1323.
- Abramson, D.H., Catalanotti, F., Brodie, S.E., Kellick, M.G., Francis, J.H., 2018a. Intravitreal chemotherapy and laser for newly visible subretinal seeds in retinoblastoma. *Ophthalmic Genet* 39, 353-356.
- Abramson, D.H., Daniels, A.B., Marr, B.P., Francis, J.H., Brodie, S.E., Dunkel, I.J., Gobin, Y.P., 2016a. Intra-Arterial Chemotherapy (Ophthalmic Artery Chemosurgery) for Group D Retinoblastoma. *PLoS One* 11, e0146582.
- Abramson, D.H., Du, T.T., Beaverson, K.L., 2002. (Neonatal) retinoblastoma in the first month of life. *Arch Ophthalmol* 120, 738-742.
- Abramson, D.H., Dunkel, I.J., Brodie, S.E., Kim, J.W., Gobin, Y.P., 2008. A phase I/II study of direct intraarterial (ophthalmic artery) chemotherapy with melphalan for intraocular retinoblastoma initial results. *Ophthalmology* 115, 1398-1404, 1404 e1391.
- Abramson, D.H., Dunkel, I.J., Brodie, S.E., Marr, B., Gobin, Y.P., 2010. Bilateral superselective ophthalmic artery chemotherapy for bilateral retinoblastoma: tandem therapy. *Arch Ophthalmol* 128, 370-372.

Abramson, D.H., Fabius, A.W., Francis, J.H., Marr, B.P., Dunkel, I.J., Brodie, S.E., Escuder, A., Gobin, Y.P., 2017a. Ophthalmic artery chemosurgery for eyes with advanced retinoblastoma. *Ophthalmic Genet* 38, 16-21.

Abramson, D.H., Fabius, A.W., Issa, R., Francis, J.H., Marr, B.P., Dunkel, I.J., Gobin, Y.P., 2015a. Advanced Unilateral Retinoblastoma: The Impact of Ophthalmic Artery Chemosurgery on Enucleation Rate and Patient Survival at MSKCC. *PLoS One* 10, e0145436.

Abramson, D.H., Frank, C.M., 1998. Second nonocular tumors in survivors of bilateral retinoblastoma: a possible age effect on radiation-related risk. *Ophthalmology* 105, 573-579; discussion 579-580.

Abramson, D.H., Frank, C.M., Chantada, G.L., de Totah, A.B., de Pifano, I.T., Ramirez, G.T., Gomez, R.T., Fandino, A.T., Tran, H.T., Madden, T.J., Dunkel, I.J., 1999a. Intraocular carboplatin concentrations following intravenous administration for human intraocular retinoblastoma. *Ophthalmic Genet* 20, 31-36.

Abramson, D.H., Frank, C.M., Dunkel, I.J., 1999b. A phase I/II study of subconjunctival carboplatin for intraocular retinoblastoma. *Ophthalmology* 106, 1947-1950.

Abramson, D.H., Frank, C.M., Susman, M., Whalen, M.P., Dunkel, I.J., Boyd, N.W., 3rd, 1998. Presenting signs of retinoblastoma. *J Pediatr* 132, 505-508.

Abramson, D.H., Gombos, D.S., 1996. The topography of bilateral retinoblastoma lesions. *Retina* 16, 232-239.

Abramson, D.H., Ji, X., Francis, J.H., Catalanotti, F., Brodie, S.E., Habib, L., 2018b. Intravitreal chemotherapy in retinoblastoma: expanded use beyond intravitreal seeds. *Br J Ophthalmol*.

Abramson, D.H., Marr, B.P., Brodie, S.E., Dunkel, I., Palioura, S., Gobin, Y.P., 2012a. Ophthalmic artery chemosurgery for less advanced intraocular retinoblastoma: five year review. *PLoS One* 7, e34120.

Abramson, D.H., Marr, B.P., Brodie, S.E., Dunkel, I.J., Lin, A., Folberg, R., Gobin, Y.P., 2012b. Intraocular hemorrhage after intra-arterial chemotherapy for retinoblastoma in sickle cell trait. *Open Ophthalmol J* 6, 1-3.

Abramson, D.H., Marr, B.P., Dunkel, I.J., Brodie, S., Zabor, E.C., Driscoll, S.J., Gobin, Y.P., 2012c. Intra-arterial chemotherapy for retinoblastoma in eyes with vitreous and/or subretinal seeding: 2-year results. *Br J Ophthalmol* 96, 499-502.

Abramson, D.H., Marr, B.P., Francis, J.H., Dunkel, I.J., Fabius, A.W., Brodie, S.E., Mondesire-Crump, I., Gobin, Y.P., 2016b. Simultaneous Bilateral Ophthalmic Artery Chemosurgery for Bilateral Retinoblastoma (Tandem Therapy). *PLoS One* 11, e0156806.

Abramson, D.H., Melson, M.R., Dunkel, I.J., Frank, C.M., 2001. Third (fourth and fifth) nonocular tumors in survivors of retinoblastoma. *Ophthalmology* 108, 1868-1876.

Abramson, D.H., Ronner, H.J., Ellsworth, R.M., 1979. Second tumors in nonirradiated bilateral retinoblastoma. *Am J Ophthalmol* 87, 624-627.

Abramson, D.H., Scheffler, A.C., 2004a. Transpupillary thermotherapy as initial treatment for small intraocular retinoblastoma: technique and predictors of success. *Ophthalmology* 111, 984-991.

Abramson, D.H., Scheffler, A.C., 2004b. Update on retinoblastoma. *Retina* 24, 828-848.

Abramson, D.H., Shields, C.L., Jabbour, P., Teixeira, L.F., Fonseca, J.R.F., Marques, M.C.P., Munier, F.L., Puccinelli, F., Hadjistilianou, T., Bracco, S., Chantada, G., Ceciliano, A., Gobin, Y.P., 2017b. Metastatic deaths in retinoblastoma patients treated with intraarterial chemotherapy (ophthalmic artery chemosurgery) worldwide. *Int J Retina Vitreous* 3, 40.

Abramson, D.H., Shields, C.L., Munier, F.L., Chantada, G.L., 2015b. Treatment of Retinoblastoma in 2015: Agreement and Disagreement. *JAMA Ophthalmol* 133, 1341-1347.

Ahnelt, P.K., 1998. The photoreceptor mosaic. *Eye (Lond)* 12 (Pt 3b), 531-540.

Ajioka, I., Dyer, M.A., 2008. A new model of tumor susceptibility following tumor suppressor gene inactivation. *Cell Cycle* 7, 735-740.

Akolekar, R., Beta, J., Picciarelli, G., Ogilvie, C., D'Antonio, F., 2015. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 45, 16-26.

Akyuz, C., Kiratli, H., Sen, H., Aydin, B., Tarlan, B., Varan, A., 2015. Intra-Arterial Chemotherapy for Retinoblastoma: A Single-Center Experience. *Ophthalmologica* 234, 227-232.

Albert, D.M., 1987. Historic review of retinoblastoma. *Ophthalmology* 94, 654-662.

Amram, A.L., Rico, G., Kim, J.W., Chintagumpala, M., Herzog, C.E., Gombos, D.S., Chevez-Barrios, P., 2017. Vitreous Seeds in Retinoblastoma: Clinicopathologic Classification and Correlation. *Ophthalmology* 124, 1540-1547.

Anagnoste, S.R., Scott, I.U., Murray, T.G., Kramer, D., Toledano, S., 2000. Rhegmatogenous retinal detachment in retinoblastoma patients undergoing chemoreduction and cryotherapy. *Am J Ophthalmol* 129, 817-819.

Ancona-Lezama, D., Dalvin, L.A., Lucio-Alvarez, J.A., Jabbour, P., Shields, C.L., 2018. OPTHALMIC VASCULAR EVENTS AFTER INTRA-ARTERIAL CHEMOTHERAPY FOR RETINOBLASTOMA: Real-World Comparison Between Primary and Secondary Treatments. *Retina*.

Andreoli, M.T., Chau, F.Y., Shapiro, M.J., Leiderman, Y.I., 2017. Epidemiological trends in 1452 cases of retinoblastoma from the Surveillance, Epidemiology, and End Results (SEER) registry. *Can J Ophthalmol* 52, 592-598.

Antoneli, C.B., Ribeiro, K.C., Steinhorst, F., Novaes, P.E., Chojniak, M.M., Malogolowkin, M., 2006. Treatment of retinoblastoma patients with chemoreduction plus local therapy: experience of the AC Camargo Hospital, Brazil. *J Pediatr Hematol Oncol* 28, 342-345.

Antoneli, C.B., Steinhorst, F., de Cassia Braga Ribeiro, K., Novaes, P.E., Chojniak, M.M., Arias, V., de Camargo, B., 2003. Extraocular retinoblastoma: a 13-year experience. *Cancer* 98, 1292-1298.

Archer, D.B., Amoaku, W.M., Kelly, G., 1993. Choroidoretinal neovascularisation following radon seed treatment of retinoblastoma in two patients. *Br J Ophthalmol* 77, 95-99.

Azar, D., Donaldson, C., Dalla-Pozza, L., 2003. Questioning the need for routine bone marrow aspiration and lumbar puncture in patients with retinoblastoma. *Clin Exp Ophthalmol* 31, 57-60.

Azar, D., Donaldson, C., Kalapesi, F., Cumming, R., 2006. Retinoblastoma in New South Wales 1975 to 2001. *J Pediatr Hematol Oncol* 28, 642-646.

Azary, S., Ganguly, A., Bunin, G.R., Lombardi, C., Park, A.S., Ritz, B., Heck, J.E., 2016. Sporadic Retinoblastoma and Parental Smoking and Alcohol Consumption before and after Conception: A Report from the Children's Oncology Group. *PLoS One* 11, e0151728.

Aziz, H.A., Kim, J.W., Munier, F.L., Berry, J.L., 2017. Acute Hemorrhagic Retinopathy following Intravitreal Melphalan Injection for Retinoblastoma: A Report of Two Cases and Technical Modifications to Enhance the Prevention of Retinal Toxicity. *Ocul Oncol Pathol* 3, 34-40.

Bader, J.L., Meadows, A.T., Zimmerman, L.E., Rorke, L.B., Voute, P.A., Champion, L.A., Miller, R.W., 1982. Bilateral retinoblastoma with ectopic intracranial retinoblastoma: trilateral retinoblastoma. *Cancer Genet Cytogenet* 5, 203-213.

- Baker, M.S., McConnell, L.K., Kleinberg, T.T., Shriver, E.M., Bilyk, J.R., Allen, R.C., 2016. Orbital sarcomas in retinoblastoma patients: recommendations for screening and treatment guidelines. *Curr Opin Ophthalmol* 27, 443-448.
- Bakhshi, S., Meel, R., Kashyap, S., Sharma, S., 2011. Bone marrow aspirations and lumbar punctures in retinoblastoma at diagnosis: correlation with IRSS staging. *J Pediatr Hematol Oncol* 33, e182-185.
- Balaguer, J., Wilson, M.W., Billups, C.A., Mancini, J., Haik, B.G., Qaddoumi, I., Khoury, J.D., Rodriguez-Galindo, C., 2009. Predictive factors of invasion in eyes with retinoblastoma enucleated after eye salvage treatments. *Pediatr Blood Cancer* 52, 351-356.
- Balmer, A., Munier, F., 2007. Differential diagnosis of leukocoria and strabismus, first presenting signs of retinoblastoma. *Clin Ophthalmol* 1, 431-439.
- Balmer, A., Munier, F., Gailloud, C., 1991. Retinoma. Case studies. *Ophthalmic Paediatr Genet* 12, 131-137.
- Barai, S., Bandopadhyaya, G.P., Raj, P., Julka, P.K., Kumar, R., Malhotra, A., Dhanpathi, H., Nainiwal, S., Haloi, A.K., 2004. Role of skeletal scintigraphy in advanced retinoblastomas. *Acta Radiol* 45, 313-316.
- Barboriak, D.P., Lee, L., Provenzale, J.M., 2001a. Lu-Serial MR imaging of pineal cysts: implications for natural history and follow-up. *AJR Am J Roentgenol* 176, 737-743.
- Barboriak, D.P., Lee, L., Provenzale, J.M., 2001b. Serial MR imaging of pineal cysts: implications for natural history and follow-up. *AJR Am J Roentgenol* 176, 737-743.
- Baroni, L.V., Sampor, C., Fandino, A., Solernou, V., Demirdjian, G., de Davila, M.T., Chantada, G.L., 2014. Anterior segment invasion in retinoblastoma: is it a risk factor for extraocular relapse? *J Pediatr Hematol Oncol* 36, e509-512.
- Bartuma, K., Pal, N., Kosek, S., Holm, S., All-Ericsson, C., 2014. A 10-year experience of outcome in chemotherapy-treated hereditary retinoblastoma. *Acta Ophthalmol* 92, 404-411.
- Batra, A., Kain, R., Kumari, M., Paul, R., Dhawan, D., Bakhshi, S., 2016a. Parents' Perspective of Quality of Life of Retinoblastoma Survivors. *Pediatr Blood Cancer* 63, 1287-1289.
- Batra, A., Kumari, M., Paul, R., Patekar, M., Dhawan, D., Bakhshi, S., 2016b. Quality of Life Assessment in Retinoblastoma: A Cross-Sectional Study of 122 Survivors from India. *Pediatr Blood Cancer* 63, 313-317.

- Baud, O., Cormier-Daire, V., Lyonnet, S., Desjardins, L., Turleau, C., Doz, F., 1999. Dysmorphic phenotype and neurological impairment in 22 retinoblastoma patients with constitutional cytogenetic 13q deletion. *Clin Genet* 55, 478-482.
- Baumal, C.R., Shields, C.L., Shields, J.A., Tasman, W.S., 1998. Surgical repair of rhegmatogenous retinal detachment after treatment for retinoblastoma. *Ophthalmology* 105, 2134-2139.
- Beck, M.N., Balmer, A., Dessing, C., Pica, A., Munier, F., 2000. First-line chemotherapy with local treatment can prevent external-beam irradiation and enucleation in low-stage intraocular retinoblastoma. *J Clin Oncol* 18, 2881-2887.
- Beck Popovic, M., Balmer, A., Maeder, P., Braganca, T., Munier, F.L., 2006. Benign pineal cysts in children with bilateral retinoblastoma: a new variant of trilateral retinoblastoma? *Pediatr Blood Cancer* 46, 755-761.
- Bellaton, E., Bertozzi, A.I., Behar, C., Chastagner, P., Brisse, H., Sainte-Rose, C., Doz, F., Desjardins, L., 2003. Neoadjuvant chemotherapy for extensive unilateral retinoblastoma. *Br J Ophthalmol* 87, 327-329.
- Benavente, C.A., Dyer, M.A., 2015. Genetically Engineered Mouse and Orthotopic Human Tumor Xenograft Models of Retinoblastoma, in: Eferl, R., Casanova, E. (Eds.), *Mouse Models of Cancer: Methods and Protocols*. Springer New York, New York, NY, pp. 307-317.
- Berry, J.L., Bechtold, M., Shah, S., Zolfaghari, E., Reid, M., Jubran, R., Kim, J.W., 2017a. Not All Seeds Are Created Equal: Seed Classification Is Predictive of Outcomes in Retinoblastoma. *Ophthalmology* 124, 1817-1825.
- Berry, J.L., Cobrinik, D., Kim, J.W., 2016a. Detection and Intraretinal Localization of an 'Invisible' Retinoblastoma Using Optical Coherence Tomography. *Ocul Oncol Pathol* 2, 148-152.
- Berry, J.L., Jubran, R., Kim, J.W., Wong, K., Bababeygy, S.R., Almarzouki, H., Lee, T.C., Murphree, A.L., 2013. Long-term outcomes of Group D eyes in bilateral retinoblastoma patients treated with chemoreduction and low-dose IMRT salvage. *Pediatr Blood Cancer* 60, 688-693.
- Berry, J.L., Jubran, R., Lee, T.C., Murphree, A.L., Lee, D., Kim, J.W., 2015. Low-Dose Chemoreduction for Infants Diagnosed with Retinoblastoma before 6 Months of Age. *Ocul Oncol Pathol* 1, 103-110.
- Berry, J.L., Kogachi, K., Aziz, H.A., McGovern, K., Zolfaghari, E., Murphree, A.L., Jubran, R., Kim, J.W., 2017b. Risk of metastasis and orbital recurrence in advanced retinoblastoma eyes treated with systemic chemoreduction versus primary enucleation. *Pediatr Blood Cancer* 64.

Berry, J.L., Kogachi, K., Jubran, R., Kim, J.W., 2018a. Loss of fundus view as an indication for secondary enucleation in retinoblastoma. *Pediatr Blood Cancer* 65.

Berry, J.L., Shih, G., Moysidis, S.N., Jubran, R., Wong, K., Lee, T.C., Murphree, A.L., Kim, J.W., 2016b. Patterns of subretinal fluid resolution in Group D eyes treated with chemoreduction: Experience from the Children's Hospital Los Angeles/University of Southern California. *Ophthalmic Genet* 37, 400-403.

Berry, J.L., Xu, L., Kooi, I., Murphree, A.L., Prabakar, R.K., Reid, M., Stachelek, K., Le, B.H.A., Welter, L., Reiser, B.J., Chevez-Barrios, P., Jubran, R., Lee, T.C., Kim, J.W., Kuhn, P., Cobrinik, D., Hicks, J., 2018b. Genomic cfDNA Analysis of Aqueous Humor in Retinoblastoma Predicts Eye Salvage: The Surrogate Tumor Biopsy for Retinoblastoma. *Mol Cancer Res* 16, 1701-1712.

Berry, J.L., Xu, L., Murphree, A.L., Krishnan, S., Stachelek, K., Zolfaghari, E., McGovern, K., Lee, T.C., Carlsson, A., Kuhn, P., Kim, J.W., Cobrinik, D., Hicks, J., 2017c. Potential of Aqueous Humor as a Surrogate Tumor Biopsy for Retinoblastoma. *JAMA Ophthalmol* 135, 1221-1230.

Bhatnagar, R., Vine, A.K., 1991. Diffuse infiltrating retinoblastoma. *Ophthalmology* 98, 1657-1661.

Bianciotto, C., Shields, C.L., Iturralde, J.C., Sarici, A., Jabbour, P., Shields, J.A., 2012. Fluorescein angiographic findings after intra-arterial chemotherapy for retinoblastoma. *Ophthalmology* 119, 843-849.

Blach, L.E., McCormick, B., Abramson, D.H., 1996. External beam radiation therapy and retinoblastoma: long-term results in the comparison of two techniques. *Int J Radiat Oncol Biol Phys* 35, 45-51.

Blach, L.E., McCormick, B., Abramson, D.H., Ellsworth, R.M., 1994. Trilateral retinoblastoma--incidence and outcome: a decade of experience. *Int J Radiat Oncol Biol Phys* 29, 729-733.

Bogenmann, E., Lochrie, M.A., Simon, M.I., 1988. Cone cell-specific genes expressed in retinoblastoma. *Science* 240, 76-78.

Bojinova, R.I., Schorderet, D.F., Addor, M.C., Gaide, A.C., Thonney, F., Pescia, G., Nenadov-Beck, M., Balmer, A., Munier, F.L., 2001. Further delineation of the facial 13q14 deletion syndrome in 13 retinoblastoma patients. *Ophthalmic Genet* 22, 11-18.

Bohm, K.J., Gobin, Y.P., Francis, J.H., McInerney, G., Dabo-Trubelja, A., Dalecki, P.H., Marr, B.P., Abramson, D.H., 2018. Choroidal infarction following ophthalmic artery chemotherapy. *Int J Retina Vitreous* 4, 16.

Bond, W.S., Akinfenwa, P.Y., Perlaky, L., Hurwitz, M.Y., Hurwitz, R.L., Chevez-Barrios, P., 2013. Tumorspheres but not adherent cells derived from retinoblastoma tumors are of malignant origin. *PLoS One* 8, e63519.

Bovey, E.H., Fernandez-Ragaz, A., Heon, E., Balmer, A., Munier, F.L., 1999. Rhegmatogenous retinal detachment after treatment of retinoblastoma. *Ophthalmic Genet* 20, 141-151.

Bowman, R.J., Mafwiri, M., Luthert, P., Luande, J., Wood, M., 2008. Outcome of retinoblastoma in east Africa. *Pediatr Blood Cancer* 50, 160-162.

Bracco, S., Leonini, S., De Francesco, S., Cioni, S., Gennari, P., Vallone, I.M., Piu, P., Galimberti, D., Romano, D.G., Caini, M., De Luca, M., Toti, P., Galluzzi, P., Hadjistilianou, T., Cerase, A., 2013. Intra-arterial chemotherapy with melphalan for intraocular retinoblastoma. *Br J Ophthalmol* 97, 1219-1221.

Brennan, R.C., Federico, S., Bradley, C., Zhang, J., Flores-Otero, J., Wilson, M., Stewart, C., Zhu, F., Guy, K., Dyer, M.A., 2011. Targeting the p53 pathway in retinoblastoma with subconjunctival Nutlin-3a. *Cancer Res* 71, 4205-4213.

Brennan, R.C., Qaddoumi, I., Billups, C.A., Free, T.L., Haik, B.G., Rodriguez-Galindo, C., Wilson, M.W., 2015. Comparison of high-risk histopathological features in eyes with primary or secondary enucleation for retinoblastoma. *Br J Ophthalmol* 99, 1366-1371.

Brennan, R.C., Qaddoumi, I., Mao, S., Wu, J., Billups, C.A., Stewart, C.F., Hoehn, M.E., Rodriguez-Galindo, C., Wilson, M.W., 2017. Ocular Salvage and Vision Preservation Using a Topotecan-Based Regimen for Advanced Intraocular Retinoblastoma. *J Clin Oncol* 35, 72-77.

Brichard, B., De Bruycker, J.J., De Potter, P., Neven, B., Vermeylen, C., Cornu, G., 2002. Combined chemotherapy and local treatment in the management of intraocular retinoblastoma. *Med Pediatr Oncol* 38, 411-415.

Brinkmann, R., Koinzer, S., Schlott, K., Ptaszynski, L., Bever, M., Baade, A., Luft, S., Miura, Y., Roider, J., Birngruber, R., 2012. Real-time temperature determination during retinal photocoagulation on patients. *J Biomed Opt* 17, 061219.

Brisse, H.J., de Graaf, P., Galluzzi, P., Cosker, K., Maeder, P., Goricke, S., Rodjan, F., de Jong, M.C., Savignoni, A., Aerts, I., Desjardins, L., Moll, A.C., Hadjistilianou, T., Toti, P., van der Valk, P., Castelijns, J.A., Sastre-Garau, X., European Retinoblastoma Imaging, C., 2015.

Assessment of early-stage optic nerve invasion in retinoblastoma using high-resolution 1.5 Tesla MRI with surface coils: a multicentre, prospective accuracy study with histopathological correlation. *Eur Radiol* 25, 1443-1452.

Broadbuss, E., Topham, A., Singh, A.D., 2009. Incidence of retinoblastoma in the USA: 1975-2004. *Br J Ophthalmol* 93, 21-23.

Brooks, H.L., Jr., Meyer, D., Shields, J.A., Balas, A.G., Nelson, L.B., Fontanesi, J., 1990. Removal of radiation-induced cataracts in patients treated for retinoblastoma. *Arch Ophthalmol* 108, 1701-1708.

Buerk, B.M., Lai, W.W., Sharma, M.C., Shapiro, M.J., 2006. Minimally invasive method for repair of rhegmatogenous retinal detachment following treatment for retinoblastoma. *Ophthalmic Surg Lasers Imaging* 37, 82-85.

Buitrago, E., Del Sole, M.J., Torbidoni, A., Fandino, A., Asprea, M., Croxatto, J.O., Chantada, G.L., Bramuglia, G.F., Schaiquevich, P., 2013. Ocular and systemic toxicity of intravitreal topotecan in rabbits for potential treatment of retinoblastoma. *Exp Eye Res* 108, 103-109.

Buitrago, E., Hocht, C., Chantada, G., Fandino, A., Navo, E., Abramson, D.H., Schaiquevich, P., Bramuglia, G.F., 2010. Pharmacokinetic analysis of topotecan after intra-vitreous injection. Implications for retinoblastoma treatment. *Exp Eye Res* 91, 9-14.

Buitrago, E., Winter, U., Williams, G., Asprea, M., Chantada, G., Schaiquevich, P., 2016. Pharmacokinetics of Melphalan After Intravitreal Injection in a Rabbit Model. *J Ocul Pharmacol Ther* 32, 230-235.

Byrne, J., Fears, T.R., Whitney, C., Parry, D.M., 1995. Survival after retinoblastoma: long-term consequences and family history of cancer. *Med Pediatr Oncol* 24, 160-165.

Canturk, S., Qaddoumi, I., Khetan, V., Ma, Z., Furmanchuk, A., Antoneli, C.B., Sultan, I., Kebudi, R., Sharma, T., Rodriguez-Galindo, C., Abramson, D.H., Chantada, G.L., 2010. Survival of retinoblastoma in less-developed countries impact of socioeconomic and health-related indicators. *Br J Ophthalmol* 94, 1432-1436.

Cao, L., Peng, B., Yao, L., Zhang, X., Sun, K., Yang, X., Yu, L., 2010. The ancient function of RB-E2F pathway: insights from its evolutionary history. *Biol Direct* 5, 55.

Carcaboso, A.M., Bramuglia, G.F., Chantada, G.L., Fandino, A.C., Chiappetta, D.A., de Davila, M.T., Rubio, M.C., Abramson, D.H., 2007. Topotecan vitreous levels after periocular or intravenous delivery in rabbits: an alternative for retinoblastoma chemotherapy. *Invest Ophthalmol Vis Sci* 48, 3761-3767.

- Carcaboso, A.M., Chiappetta, D.A., Opezzo, J.A., Hocht, C., Fandino, A.C., Croxatto, J.O., Rubio, M.C., Sosnik, A., Abramson, D.H., Bramuglia, G.F., Chantada, G.L., 2010. Episcleral implants for topotecan delivery to the posterior segment of the eye. *Invest Ophthalmol Vis Sci* 51, 2126-2134.
- Cassoux, N., Aerts, I., Lumbroso-Le Rouic, L., Freneaux, P., Desjardins, L., 2017. Eye Salvage with Combination of Intravitreal and Intracameral Melphalan Injection for Recurrent Retinoblastoma with Anterior Chamber Involvement: Report of a Case. *Ocul Oncol Pathol* 3, 129-132.
- Castera, L., Sabbagh, A., Dehainault, C., Michaux, D., Mansuet-Lupo, A., Patillon, B., Lamar, E., Aerts, I., Lumbroso-Le Rouic, L., Couturier, J., Stoppa-Lyonnet, D., Gauthier-Villars, M., Houdayer, C., 2010. MDM2 as a modifier gene in retinoblastoma. *J Natl Cancer Inst* 102, 1805-1808.
- Cavenee, W.K., Dryja, T.P., Phillips, R.A., Benedict, W.F., Godbout, R., Gallie, B.L., Murphree, A.L., Strong, L.C., White, R.L., 1983. Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. *Nature* 305, 779-784.
- Cebulla, C.M., Alegret, A., Ehli, F.J., Davis, R.P., 2nd, Hess, D.J., Murray, T.G., Echography Study, G., 2009. Echographic localization of periocular Carboplatin for treatment of advanced retinoblastoma. *Retin Cases Brief Rep* 3, 4-7.
- Chan, M.P., Hungerford, J.L., Kingston, J.E., Plowman, P.N., 2009. Salvage external beam radiotherapy after failed primary chemotherapy for bilateral retinoblastoma: rate of eye and vision preservation. *Br J Ophthalmol* 93, 891-894.
- Chantada, G., Doz, F., Antoneli, C.B., Grundy, R., Clare Stannard, F.F., Dunkel, I.J., Grabowski, E., Leal-Leal, C., Rodriguez-Galindo, C., Schwartzman, E., Popovic, M.B., Kremens, B., Meadows, A.T., Zucker, J.M., 2006a. A proposal for an international retinoblastoma staging system. *Pediatr Blood Cancer* 47, 801-805.
- Chantada, G., Fandino, A., Davila, M.T., Manzitti, J., Raslawski, E., Casak, S., Schwartzman, E., 2004. Results of a prospective study for the treatment of retinoblastoma. *Cancer* 100, 834-842.
- Chantada, G., Luna-Fineman, S., Sitorus, R.S., Kruger, M., Israels, T., Leal-Leal, C., Bakhshi, S., Qaddoumi, I., Abramson, D.H., Doz, F., Committee, S.-P.G.-I.R.G.W., 2013. SIOP-PODC recommendations for graduated-intensity treatment of retinoblastoma in developing countries. *Pediatr Blood Cancer* 60, 719-727.

Chantada, G.L., Dunkel, I.J., Antoneli, C.B., de Davila, M.T., Arias, V., Beaverson, K., Fandino, A.C., Chojniak, M., Abramson, D.H., 2007. Risk factors for extraocular relapse following enucleation after failure of chemoreduction in retinoblastoma. *Pediatr Blood Cancer* 49, 256-260.

Chantada, G.L., Fandino, A.C., Carcaboso, A.M., Lagomarsino, E., de Davila, M.T., Gutter, M.R., Rose, A.B., Manzitti, J., Bramuglia, G.F., Abramson, D.H., 2009. A phase I study of periocular topotecan in children with intraocular retinoblastoma. *Invest Ophthalmol Vis Sci* 50, 1492-1496.

Chantada, G.L., Fandino, A.C., Gutter, M.R., Raslawski, E.C., Dominguez, J.L., Manzitti, J., de Davila, M.T., Zubizarreta, P., Scopinaro, M., 2010. Results of a prospective study for the treatment of unilateral retinoblastoma. *Pediatr Blood Cancer* 55, 60-66.

Chantada, G.L., Fandino, A.C., Raslawski, E.C., Manzitti, J., de Davila, M.T., Casak, S.J., Scopinaro, M.J., Schwartzman, E., 2005. Experience with chemoreduction and focal therapy for intraocular retinoblastoma in a developing country. *Pediatr Blood Cancer* 44, 455-460.

Chantada, G.L., Fandino, A.C., Schwartzman, E., Raslawski, E., Schaiquevich, P., Manzitti, J., 2014. Impact of chemoreduction for conservative therapy for retinoblastoma in Argentina. *Pediatr Blood Cancer* 61, 821-826.

Chantada, G.L., Qaddoumi, I., Canturk, S., Khetan, V., Ma, Z., Kimani, K., Yeniad, B., Sultan, I., Sitorus, R.S., Tacyildiz, N., Abramson, D.H., 2011. Strategies to manage retinoblastoma in developing countries. *Pediatr Blood Cancer* 56, 341-348.

Chantada, G.L., Rossi, J., Casco, F., Fandino, A., Scopinaro, M., de Davila, M.T., Abramson, D.H., 2006b. An aggressive bone marrow evaluation including immunocytology with GD2 for advanced retinoblastoma. *J Pediatr Hematol Oncol* 28, 369-373.

Chaudhry, S., Onadim, Z., Sagoo, M.S., Reddy, M.A., 2018. THE RECOGNITION OF CAVITARY RETINOBLASTOMA TUMORS: Implications for Management and Genetic Analysis. *Retina* 38, 782-787.

Chaussade, A., Millot, G., Wells, C., Brisse, H., Lae, M., Savignoni, A., Desjardins, L., Dendale, R., Doz, F., Aerts, I., Jimenez, I., Cassoux, N., Stoppa Lyonnet, D., Gauthier Villars, M., Houdayer, C., 2018. Correlation between RB1 germline mutations and second primary malignancies in hereditary retinoblastoma patients treated with external beam radiotherapy. *Eur J Med Genet*.

Chawla, B., Hasan, F., Azad, R., Seth, R., Upadhyay, A.D., Pathy, S., Pandey, R.M., 2016. Clinical presentation and survival of retinoblastoma in Indian children. *Br J Ophthalmol* 100, 172-178.

Chen, D., Livne-bar, I., Vanderluit, J.L., Slack, R.S., Agochiya, M., Bremner, R., 2004. Cell-specific effects of RB or RB/p107 loss on retinal development implicate an intrinsically death-resistant cell-of-origin in retinoblastoma. *Cancer Cell* 5, 539-551.

Chen, M., Jiang, H., Zhang, J., Shen, G., Jiang, Y., Li, H., Liu, Z., 2017. Outcome of intra-arterial chemotherapy for retinoblastoma and its influencing factors: a retrospective study. *Acta Ophthalmol* 95, 613-618.

Chevez-Barrios, P., Chintagumpala, M., Mieler, W., Paysse, E., Boniuk, M., Kozinetz, C., Hurwitz, M.Y., Hurwitz, R.L., 2005. Response of retinoblastoma with vitreous tumor seeding to adenovirus-mediated delivery of thymidine kinase followed by ganciclovir. *J Clin Oncol* 23, 7927-7935.

Chevez-Barrios, P., Hurwitz, M.Y., Louie, K., Marcus, K.T., Holcombe, V.N., Schafer, P., Aguilar-Cordova, C.E., Hurwitz, R.L., 2000. Metastatic and nonmetastatic models of retinoblastoma. *Am J Pathol* 157, 1405-1412.

Chhablani, J., Romanzo, A., Balmer, A., Pica, A., Gaillard, M.C., Cozza, R., Moeckli, R., Munier, F.L., 2010. (106)Ruthenium brachytherapy for ciliary recurrence with supraciliary effusion in retinoblastoma. *Ophthalmic Genet* 31, 190-192.

Chiang, B., Jung, J.H., Prausnitz, M.R., 2018. The suprachoroidal space as a route of administration to the posterior segment of the eye. *Adv Drug Deliv Rev*.

Chung, J., Turaka, K., Shields, C.L., 2010. Retinocytoma shows lack of response to chemoreduction. *J Pediatr Ophthalmol Strabismus* 47 Online, e1-3.

Chung, S.E., Sa, H.S., Koo, H.H., Yoo, K.H., Sung, K.W., Ham, D.I., 2008. Clinical manifestations and treatment of retinoblastoma in Korea. *Br J Ophthalmol* 92, 1180-1184.

Clarke, A.R., Maandag, E.R., van Roon, M., van der Lugt, N.M., van der Valk, M., Hooper, M.L., Berns, A., te Riele, H., 1992. Requirement for a functional Rb-1 gene in murine development. *Nature* 359, 328-330.

Clavel, C.M., Nowak-Sliwinska, P., Păunescu, E., Dyson, P.J., 2015. Thermoresponsive fluorinated small-molecule drugs: a new concept for efficient localized chemotherapy. *MedChemComm* 6, 2054-2062.

Cobrinik, D., 2013. Learning about retinoblastoma from mouse models that missed, in: Martinez-Murillo, R., Martinez, A. (Eds.), *Animal Models of Brain Tumors*. Springer, New York, pp. 141-152.

- Cobrinik, D., 2015. Retinoblastoma Progression. *EBioMedicine* 2, 623-624.
- Cohen, V.M., Shields, C.L., Furuta, M., Shields, J.A., 2008. Vitreous seeding from retinal astrocytoma in three cases. *Retina* 28, 884-888.
- Conkrite, K., Sundby, M., Mu, D., Mukai, S., MacPherson, D., 2012. Cooperation between Rb and Arf in suppressing mouse retinoblastoma. *J Clin Invest* 122, 1726-1733.
- Cornish, E.E., Xiao, M., Yang, Z., Provis, J.M., Hendrickson, A.E., 2004. The role of opsin expression and apoptosis in determination of cone types in human retina. *Exp Eye Res* 78, 1143-1154.
- Corson, T.W., Gallie, B.L., 2007. One hit, two hits, three hits, more? Genomic changes in the development of retinoblastoma. *Genes Chromosomes Cancer* 46, 617-634.
- Cozza, R., De Ioris, M.A., Ilari, I., Devito, R., Fidani, P., De Sio, L., Demelas, F., Romanzo, A., Donfrancesco, A., 2009. Metastatic retinoblastoma: single institution experience over two decades. *Br J Ophthalmol* 93, 1163-1166.
- Curcio, C.A., Sloan, K.R., Kalina, R.E., Hendrickson, A.E., 1990. Human photoreceptor topography. *J Comp Neurol* 292, 497-523.
- Daicker, B., 1972. Anatomie und Pathologie der menschlichen retino-ziliaren Fundusperipherie, Anatomie und Pathologie der menschlichen retino-ziliaren Fundusperipherie. Karger Publishers, pp. I-VIII.
- Dalvin, L.A., Ancona-Lezama, D., Lucio-Alvarez, J.A., Masoomian, B., Jabbour, P., Shields, C.L., 2018. Ophthalmic Vascular Events after Primary Unilateral Intra-arterial Chemotherapy for Retinoblastoma in Early and Recent Eras. *Ophthalmology* 125, 1803-1811.
- Daniels, A.B., Froehler, M.T., Nunnally, A.H., Pierce, J.M., Bozic, I., Stone, C.A., Santapuram, P.R., Tao, Y.K., Boyd, K.L., Himmel, L.E., Chen, S.C., Du, L., Friedman, D.L., Richmond, A., 2019. Rabbit Model of Intra-Arterial Chemotherapy Toxicity Demonstrates Retinopathy and Vasculopathy Related to Drug and Dose, Not Procedure or Approach. *Invest Ophthalmol Vis Sci* 60, 954-964.
- Daniels, A.B., Froehler, M.T., Pierce, J.M., Nunnally, A.H., Calcutt, M.W., Bridges, T.M., LaNeve, D.C., Williams, P.E., Boyd, K.L., Reyzer, M.L., Lindsley, C.W., Friedman, D.L., Richmond, A., 2018. Pharmacokinetics, Tissue Localization, Toxicity, and Treatment Efficacy in the First Small Animal (Rabbit) Model of Intra-Arterial Chemotherapy for Retinoblastoma. *Invest Ophthalmol Vis Sci* 59, 446-454.

Daruich, A.L., Moulin, A.P., Tran, H.V., Matet, A., Munier, F.L., 2017. SUBFOVEAL NODULE IN COATS' DISEASE: Toward an Updated Classification Predicting Visual Prognosis. *Retina* 37, 1591-1598.

De Francesco, S., Pichi, F., Lembo, A., Mastrangelo, D., Paradiso, A., De Luca, M., Toti, P., Nucci, P., Hadjistilianou, T., 2014. Massive anterior chamber involvement after intra-arterial chemotherapy for retinoblastoma: ultrasound biomicroscopy and histopathology. *Int Ophthalmol* 34, 967-970.

de Graaf, P., Goricke, S., Rodjan, F., Galluzzi, P., Maeder, P., Castelijns, J.A., Brisse, H.J., 2012a. Guidelines for imaging retinoblastoma: imaging principles and MRI standardization. *Pediatric Radiology* 42, 2-14.

de Graaf, P., Goricke, S., Rodjan, F., Galluzzi, P., Maeder, P., Castelijns, J.A., Brisse, H.J., European Retinoblastoma Imaging, C., 2012b. Guidelines for imaging retinoblastoma: imaging principles and MRI standardization. *Pediatr Radiol* 42, 2-14.

De Ioris, M.A., Valente, P., Randisi, F., Buzzonetti, L., Carai, A., Cozza, R., Del Bufalo, F., Romano, A., Angioni, A., Cacchione, A., Bernardi, B., Mastronuzzi, A., 2014. Baseline central nervous system magnetic resonance imaging in early detection of trilateral retinoblastoma: pitfalls in the diagnosis of pineal gland lesions. *Anticancer Res* 34, 7449-7454.

de Jong, M.C., de Graaf, P., Brisse, H.J., Galluzzi, P., Goricke, S.L., Moll, A.C., Munier, F.L., Popovic, M.B., Moulin, A.P., Binaghi, S., Castelijns, J.A., Maeder, P., European Retinoblastoma Imaging, C., 2015a. The potential of 3T high-resolution magnetic resonance imaging for diagnosis, staging, and follow-up of retinoblastoma. *Surv Ophthalmol* 60, 346-355.

de Jong, M.C., Kors, W.A., de Graaf, P., Castelijns, J.A., Kivela, T., Moll, A.C., 2014a. Trilateral retinoblastoma: a systematic review and meta-analysis. *Lancet Oncol* 15, 1157-1167.

de Jong, M.C., Kors, W.A., de Graaf, P., Castelijns, J.A., Moll, A.C., Kivela, T., 2015b. The Incidence of Trilateral Retinoblastoma: A Systematic Review and Meta-Analysis. *Am J Ophthalmol* 160, 1116-1126 e1115.

de Jong, M.C., Moll, A.C., Goricke, S., van der Valk, P., Kors, W.A., Castelijns, J.A., de Graaf, P., 2014b. lu-From a Suspicious Cystic Pineal Gland to Pineoblastoma in a Patient with Familial Unilateral Retinoblastoma. *Ophthalmic Genet*, 1-3.

De la Huerta, I., Seider, M.I., Hetts, S.W., Damato, B.E., 2016. Delayed Cerebral Infarction Following Intra-arterial Chemotherapy for Retinoblastoma. *JAMA Ophthalmol* 134, 712-714.

Del Amo, E.M., Rimpela, A.K., Heikkinen, E., Kari, O.K., Ramsay, E., Lajunen, T., Schmitt, M., Pelkonen, L., Bhattacharya, M., Richardson, D., Subrizi, A., Turunen, T., Reinisalo, M., Itkonen, J., Toropainen, E., Casteleijn, M., Kidron, H., Antopolsky, M., Vellonen, K.S., Ruponen, M., Urtti, A., 2017. Pharmacokinetic aspects of retinal drug delivery. *Prog Retin Eye Res* 57, 134-185.

Demirci, H., Shields, C.L., Meadows, A.T., Shields, J.A., 2005. Long-term visual outcome following chemoreduction for retinoblastoma. *Arch Ophthalmol* 123, 1525-1530.

Dimaras, H., Corson, T.W., Cobrinik, D., White, A., Zhao, J., Munier, F.L., Abramson, D.H., Shields, C.L., Chantada, G.L., Njuguna, F., Gallie, B.L., 2015. Retinoblastoma. *Nat Rev Dis Primers* 1, 15021.

Dimaras, H., Khetan, V., Halliday, W., Orlic, M., Prigoda, N.L., Piovesan, B., Marrano, P., Corson, T.W., Eagle, R.C., Jr., Squire, J.A., Gallie, B.L., 2008a. Loss of RB1 induces non-proliferative retinoma; increasing genomic instability correlates with progression to retinoblastoma. *Hum Mol Genet*.

Dimaras, H., Khetan, V., Halliday, W., Orlic, M., Prigoda, N.L., Piovesan, B., Marrano, P., Corson, T.W., Eagle, R.C., Jr., Squire, J.A., Gallie, B.L., 2008b. Loss of RB1 induces non-proliferative retinoma: increasing genomic instability correlates with progression to retinoblastoma. *Hum Mol Genet* 17, 1363-1372.

Ditta, L.C., Choudhri, A.F., Tse, B.C., Landers, M.M., Haik, B.G., Steinle, J.J., Williams, J.S., Wilson, M.W., 2012. Validating a nonhuman primate model of super-selective intraophthalmic artery chemotherapy: comparing ophthalmic artery diameters. *Invest Ophthalmol Vis Sci* 53, 7791-7794.

DIVISION, U.N.D.P., 2017. United Nations, Department of Economic and Social Affairs, Population Division, 2017, in: Revision., W.P.P.T. (Ed.), <https://esa.un.org/unpd/wpp/>.

Dommering, C.J., Marees, T., van der Hout, A.H., Imhof, S.M., Meijers-Heijboer, H., Ringens, P.J., van Leeuwen, F.E., Moll, A.C., 2012. RB1 mutations and second primary malignancies after hereditary retinoblastoma. *Fam Cancer* 11, 225-233.

Doz, F., Neuenschwander, S., Plantaz, D., Courbon, B., Gentet, J.C., Bouffet, E., Mosseri, V., Vannier, J.P., Mechinaud, F., Desjardins, L., et al., 1995. Etoposide and carboplatin in extraocular retinoblastoma: a study by the Societe Francaise d'Oncologie Pediatrique. *J Clin Oncol* 13, 902-909.

Draper, G.J., Sanders, B.M., Brownbill, P.A., Hawkins, M.M., 1992. Patterns of risk of hereditary retinoblastoma and applications to genetic counselling. *Br J Cancer* 66, 211-219.

Dryja, T.P., Rapaport, J., McGee, T.L., Nork, T.M., Schwartz, T.L., 1993. Molecular etiology of low-penetrance retinoblastoma in two pedigrees. *Am J Hum Genet* 52, 1122-1128.

Dunkel, I.J., Chan, H.S., Jubran, R., Chantada, G.L., Goldman, S., Chintagumpala, M., Khakoo, Y., Abramson, D.H., 2010a. High-dose chemotherapy with autologous hematopoietic stem cell rescue for stage 4B retinoblastoma. *Pediatr Blood Cancer* 55, 149-152.

Dunkel, I.J., Jubran, R.F., Gururangan, S., Chantada, G.L., Finlay, J.L., Goldman, S., Khakoo, Y., O'Brien, J.M., Orjuela, M., Rodriguez-Galindo, C., Souweidane, M.M., Abramson, D.H., 2010b. Trilateral retinoblastoma: potentially curable with intensive chemotherapy. *Pediatr Blood Cancer* 54, 384-387.

Dunkel, I.J., Khakoo, Y., Kernan, N.A., Gershon, T., Gilheeney, S., Lyden, D.C., Wolden, S.L., Orjuela, M., Gardner, S.L., Abramson, D.H., 2010c. Intensive multimodality therapy for patients with stage 4a metastatic retinoblastoma. *Pediatr Blood Cancer* 55, 55-59.

Dunkel, I.J., Krailo, M.D., Chantada, G.L., Banerjee, A., Abouelnaga, S., Buchsbaum, J., Merchant, T.E., Granger, M., Jubran, R.F., Kellick, M., Weinstein, J., Abramson, D.H., Rodriguez-Galindo, C., Chintagumpala, M.M., 2017. Intensive multi-modality therapy for extra-ocular retinoblastoma (RB): A Children's Oncology Group (COG) trial (ARET0321). *Journal of Clinical Oncology* 35, 10506-10506.

Dunkel, I.J., Shi, W., Salvaggio, K., Marr, B.P., Brodie, S.E., Gobin, Y.P., Abramson, D.H., 2014. Risk factors for severe neutropenia following intra-arterial chemotherapy for intra-ocular retinoblastoma. *PLoS One* 9, e108692.

Eagle, R.C., Jr., 2009. High-risk features and tumor differentiation in retinoblastoma: a retrospective histopathologic study. *Arch Pathol Lab Med* 133, 1203-1209.

Eagle, R.C., Jr., Shields, C.L., Bianciotto, C., Jabbour, P., Shields, J.A., 2011. Histopathologic observations after intra-arterial chemotherapy for retinoblastoma. *Arch Ophthalmol* 129, 1416-1421.

Eagle, R.C., Jr., Shields, J.A., Donoso, L., Milner, R.S., 1989. Malignant transformation of spontaneously regressed retinoblastoma, retinoma/retinocytoma variant. *Ophthalmology* 96, 1389-1395.

Edelhauser, H.F., Rowe-Rendleman, C.L., Robinson, M.R., Dawson, D.G., Chader, G.J., Grossniklaus, H.E., Rittenhouse, K.D., Wilson, C.G., Weber, D.A., Kuppermann, B.D., Csaky, K.G., Olsen, T.W., Kompella, U.B., Holers, V.M., Hageman, G.S., Gilger, B.C., Campochiaro, P.A., Whitcup, S.M., Wong, W.T., 2010. Ophthalmic drug delivery systems for the treatment of

retinal diseases: basic research to clinical applications. *Invest Ophthalmol Vis Sci* 51, 5403-5420.

Eisner, G., 1973. *Biomicroscopy of the peripheral fundus: an atlas and textbook*. Springer.

Ellsworth, R.M., 1969. The practical management of retinoblastoma. *Trans Am Ophthalmol Soc* 67, 462-534.

Eloy, P., Dehainault, C., Sefta, M., Aerts, I., Doz, F., Cassoux, N., Lumbroso le Rouic, L., Stoppa-Lyonnet, D., Radvanyi, F., Millot, G.A., Gauthier-Villars, M., Houdayer, C., 2016. A Parent-of-Origin Effect Impacts the Phenotype in Low Penetrance Retinoblastoma Families Segregating the c.1981C>T/p.Arg661Trp Mutation of RB1. *PLoS Genet* 12, e1005888.

Eng, C., Li, F.P., Abramson, D.H., Ellsworth, R.M., Wong, F.L., Goldman, M.B., Seddon, J., Tarbell, N., Boice, J.D., Jr., 1993. Mortality from second tumors among long-term survivors of retinoblastoma. *J Natl Cancer Inst* 85, 1121-1128.

Epstein, J.A., Shields, C.L., Shields, J.A., 2003. Trends in the management of retinoblastoma: evaluation of 1,196 consecutive eyes during 1974 to 2001. *J Pediatr Ophthalmol Strabismus* 40, 196-203; quiz 217-198.

Ericson, L.A., Rosengren, B.H., 1961. Present therapeutic resources in retinoblastoma. *Acta Ophthalmol (Copenh)* 39, 569-576.

Fabian, I.D., Naeem, Z., Stacey, A.W., Chowdhury, T., Duncan, C., Reddy, M.A., Sagoo, M.S., 2017a. Long-term Visual Acuity, Strabismus, and Nystagmus Outcomes Following Multimodality Treatment in Group D Retinoblastoma Eyes. *Am J Ophthalmol* 179, 137-144.

Fabian, I.D., Puccinelli, F., Gaillard, M.C., Beck-Popovic, M., Munier, F.L., 2017b. Diagnosis and management of secondary epipapillary retinoblastoma. *Br J Ophthalmol* 101, 1412-1418.

Fabian, I.D., Stacey, A.W., Chowdhury, T., Duncan, C., Karaa, E.K., Scheimberg, I., Reddy, M.A., Sagoo, M.S., 2017c. High-Risk Histopathology Features in Primary and Secondary Enucleated International Intraocular Retinoblastoma Classification Group D Eyes. *Ophthalmology* 124, 851-858.

Fabian, I.D., Stacey, A.W., Naeem, Z., Onadim, Z., Chowdhury, T., Duncan, C., Sagoo, M.S., Reddy, M.A., 2018. Strabismus in retinoblastoma survivors with long-term follow-up. *J AAPOS* 22, 276 e271-276 e277.

Fernandes, A.G., Pollock, B.D., Rabito, F.A., 2018. Retinoblastoma in the United States: A 40-Year Incidence and Survival Analysis. *J Pediatr Ophthalmol Strabismus* 55, 182-188.

- Fernandes, A.G.P., B.D; Rabito, F.A., 2018. Retinoblastoma in the United States: A 40-year incidence and survival analysis. *J Pediatr Ophthalmol Strabismus* 55, 182-1888.
- Fernandez, M.P., Al-Holou, S.N., Fischer, O., Murray, T., Harbour, J.W., Dubovy, S.R., Berrocal, A.M., 2017. Fluorescein angiography findings in diffuse retinoblastoma: two case reports with clinicopathologic correlation. *J AAPOS* 21, 337-339 e332.
- Fischer, C., Petriccione, M., Vitolano, S., Guarini, E., Davis, M.E., Dunkel, I.J., 2017. The Effect of Ophthalmic Artery Chemosurgery on Immune Function in Retinoblastoma Patients: A Single Institution Retrospective Analysis. *J Pediatr Hematol Oncol* 39, 555-559.
- Fledelius, H.C., Christensen, A.C., 1996. Reappraisal of the human ocular growth curve in fetal life, infancy, and early childhood. *Br J Ophthalmol* 80, 918-921.
- Fletcher, O., Easton, D., Anderson, K., Gilham, C., Jay, M., Peto, J., 2004. Lifetime risks of common cancers among retinoblastoma survivors. *J Natl Cancer Inst* 96, 357-363.
- Flexner, S., 1891. A peculiar glioma (neuroepithelioma?) of the retina. *Bull Johns Hopkins Hosp* 2, 115-119.
- Fluhs, D., Anastassiou, G., Wening, J., Sauerwein, W., Bornfeld, N., 2004. The design and the dosimetry of bi-nuclide radioactive ophthalmic applicators. *Med Phys* 31, 1481-1488.
- Fontanesi, J., Pratt, C.B., Kun, L.E., Hustu, H.O., Coffey, D., Meyer, D., 1996. Treatment outcome and dose-response relationship in infants younger than 1 year treated for retinoblastoma with primary irradiation. *Med Pediatr Oncol* 26, 297-304.
- Foos, R.Y., 1972. Vitreoretinal juncture; topographical variations. *Invest Ophthalmol* 11, 801-808.
- Foster, B.S., Mukai, S., 1996. Intraocular retinoblastoma presenting as ocular and orbital inflammation. *Int Ophthalmol Clin* 36, 153-160.
- Francis, J.H., Abramson, D.H., Brodie, S.E., Marr, B.P., 2013a. Indocyanine green enhanced transpupillary thermotherapy in combination with ophthalmic artery chemosurgery for retinoblastoma. *Br J Ophthalmol* 97, 164-168.
- Francis, J.H., Abramson, D.H., Gaillard, M.C., Marr, B.P., Beck-Popovic, M., Munier, F.L., 2015a. The classification of vitreous seeds in retinoblastoma and response to intravitreal melphalan. *Ophthalmology* 122, 1173-1179.

Francis, J.H., Abramson, D.H., Gobin, Y.P., Marr, B.P., Tendler, I., Brodie, S.E., Dunkel, I.J., 2015b. Efficacy and toxicity of second-course ophthalmic artery chemosurgery for retinoblastoma. *Ophthalmology* 122, 1016-1022.

Francis, J.H., Abramson, D.H., Ji, X., Shields, C.L., Teixeira, L.F., Scheffler, A.C., Cassoux, N., Hadjistilianou, D., Berry, J.L., Frenkel, S., Munier, F.L., 2017a. Risk of Extraocular Extension in Eyes With Retinoblastoma Receiving Intravitreal Chemotherapy. *JAMA Ophthalmol* 135, 1426-1429.

Francis, J.H., Barker, C.A., Wolden, S.L., McCormick, B., Segal, K., Cohen, G., Gobin, Y.P., Marr, B.P., Brodie, S.E., Dunkel, I.J., Abramson, D.H., 2013b. Salvage/adjuvant brachytherapy after ophthalmic artery chemosurgery for intraocular retinoblastoma. *Int J Radiat Oncol Biol Phys* 87, 517-523.

Francis, J.H., Brodie, S.E., Marr, B., Zabor, E.C., Mondesire-Crump, I., Abramson, D.H., 2017b. Efficacy and toxicity of intravitreal chemotherapy for retinoblastoma: four-year experience. *Ophthalmology* 124, 488-495.

Francis, J.H., Gobin, Y.P., Nagiel, A., Dunkel, I.J., Kucine, N., Marr, B.P., Brodie, S.E., Abramson, D.H., 2012. Thrombophilia in patients with retinoblastoma receiving ophthalmic artery chemosurgery. *Arch Ophthalmol* 130, 1605-1608.

Francis, J.H., Iyer, S., Gobin, Y.P., Brodie, S.E., Abramson, D.H., 2017c. Retinoblastoma Vitreal Seed Clouds (Class 3): A Comparison of Treatment with Ophthalmic Artery Chemosurgery with or without Intravitreal and Periocular Chemotherapy. *Ophthalmology* 124, 1548-1555.

Francis, J.H., Levin, A.M., Zabor, E.C., Gobin, Y.P., Abramson, D.H., 2018a. Ten-year experience with ophthalmic artery chemosurgery: Ocular and recurrence-free survival. *PLoS One* 13, e0197081.

Francis, J.H., Marr, B.P., Abramson, D.H., 2016. Classification of Vitreal Seeds in Retinoblastoma: Correlations with Patient, Tumor, and Treatment Characteristics. *Ophthalmology* 123, 1601-1605.

Francis, J.H., Marr, B.P., Brodie, S.E., Abramson, D.H., 2015c. Anterior Ocular Toxicity of Intravitreal Melphalan for Retinoblastoma. *JAMA Ophthalmol* 133, 1459-1463.

Francis, J.H., Roosip, N., Levin, A.M., Brodie, S.E., Dunkel, I.J., Gobin, Y.P., Abramson, D.H., 2018b. Current Treatment of Bilateral Retinoblastoma: The Impact of Intraarterial and Intravitreal Chemotherapy. *Neoplasia* 20, 757-763.

- Francis, J.H., Schaiquevich, P., Buitrago, E., Del Sole, M.J., Zapata, G., Croxatto, J.O., Marr, B.P., Brodie, S.E., Berra, A., Chantada, G.L., Abramson, D.H., 2014. Local and systemic toxicity of intravitreal melphalan for vitreous seeding in retinoblastoma: a preclinical and clinical study. *Ophthalmology* 121, 1810-1817.
- Friedman, D.N., Lis, E., Sklar, C.A., Oeffinger, K.C., Reppucci, M., Fleischut, M.H., Francis, J.H., Marr, B., Abramson, D.H., Dunkel, I.J., 2014. Whole-body magnetic resonance imaging (WB-MRI) as surveillance for subsequent malignancies in survivors of hereditary retinoblastoma: a pilot study. *Pediatr Blood Cancer* 61, 1440-1444.
- Friedrich, P., Itriago, E., Rodriguez-Galindo, C., Ribeiro, K., 2017. Racial and Ethnic Disparities in the Incidence of Pediatric Extracranial Embryonal Tumors. *J Natl Cancer Inst* 109.
- Friend, S.H., Bernards, R., Rogelj, S., Weinberg, R.A., Rapaport, J.M., Albert, D.M., Dryja, T.P., 1986. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature* 323, 643-646.
- Fugaro, M.N., Kiupel, M., Montiani-Ferreira, F., Hawkins, J.F., Janovitz, E.B., 2005. Retinoblastoma in the eye of a llama (*Llama glama*). *Vet Ophthalmol* 8, 287-290.
- Funes, S., Sampor, C., Villasante, F., Fandino, A., Manzitti, J., Sgroi, M., Neira, P., Peralta, L., Lagomarsino, E., Schaiquevich, P., Ceciliano, A., Chantada, G.L., 2018. Feasibility and results of an intraarterial chemotherapy program for the conservative treatment of retinoblastoma in Argentina. *Pediatr Blood Cancer* 65, e27086.
- Fung, Y.K., Murphree, A.L., T'Ang, A., Qian, J., Hinrichs, S.H., Benedict, W.F., 1987. Structural evidence for the authenticity of the human retinoblastoma gene. *Science* 236, 1657-1661.
- Gaillard, M.C., Houghton, S., Stathopoulos, C., Munier, F.L., 2018. OCT-guided management of subclinical recurrent retinoblastoma. *Ophthalmic Genet* 39, 338-343.
- Gaillard, M.C., Mataftsi, A., Balmer, A., Houghton, S., Munier, F.L., 2014. ranibizumab in the management of advanced Coats disease Stages 3B and 4: long-term outcomes. *Retina* 34, 2275-2281.
- Gallie, B.L., Budning, A., DeBoer, G., Thiessen, J.J., Koren, G., Verjee, Z., Ling, V., Chan, H.S., 1996. Chemotherapy with focal therapy can cure intraocular retinoblastoma without radiotherapy. *Arch Ophthalmol* 114, 1321-1328.
- Gallie, B.L., Campbell, C., Devlin, H., Duckett, A., Squire, J.A., 1999. Developmental basis of retinal-specific induction of cancer by RB mutation. *Cancer Res* 59, 1731s-1735s.

- Gallie, B.L., Phillips, R.A., Ellsworth, R.M., Abramson, D.H., 1982. Significance of retinoma and phthisis bulbi for retinoblastoma. *Ophthalmology* 89, 1393-1399.
- Gatta, G., Ferrari, A., Stiller, C.A., Pastore, G., Bisogno, G., Trama, A., Capocaccia, R., Group, R.W., 2012. Embryonal cancers in Europe. *Eur J Cancer* 48, 1425-1433.
- Gerrish, A., Stone, E., Clokie, S., Ainsworth, J.R., Jenkinson, H., McCalla, M., Hitchcott, C., Colmenero, I., Allen, S., Parulekar, M., Cole, T., 2019. Non-invasive diagnosis of retinoblastoma using cell-free DNA from aqueous humour. *Br J Ophthalmol*. [Epub ahead of print]
- Ghassemi, F., Amoli, F.A., 2014. Pathological findings in enucleated eyes after intravitreal melphalan injection. *Int Ophthalmol* 34, 533-540.
- Ghassemi, F., Rahmanikhah, E., Roohipoor, R., Karkhaneh, R., Faegh, A., 2013. Regression patterns in treated retinoblastoma with chemotherapy plus focal adjuvant therapy. *Pediatr Blood Cancer* 60, 599-604.
- Ghassemi, F., Shields, C.L., 2012. Intravitreal melphalan for refractory or recurrent vitreous seeding from retinoblastoma. *Arch Ophthalmol* 130, 1268-1271.
- Ghassemi, F., Shields, C.L., Ghadimi, H., Khodabandeh, A., Roohipoor, R., 2014. Combined intravitreal melphalan and topotecan for refractory or recurrent vitreous seeding from retinoblastoma. *JAMA Ophthalmol* 132, 936-941.
- Gobin, Y.P., Dunkel, I.J., Marr, B.P., Brodie, S.E., Abramson, D.H., 2011. Intra-arterial chemotherapy for the management of retinoblastoma: four-year experience. *Arch Ophthalmol* 129, 732-737.
- Gobin, Y.P., Dunkel, I.J., Marr, B.P., Francis, J.H., Brodie, S.E., Abramson, D.H., 2012. Combined, sequential intravenous and intra-arterial chemotherapy (bridge chemotherapy) for young infants with retinoblastoma. *PLoS One* 7, e44322.
- Goddard, A.G., Kingston, J.E., Hungerford, J.L., 1999. Delay in diagnosis of retinoblastoma: risk factors and treatment outcome. *Br J Ophthalmol* 83, 1320-1323.
- Golabchi, K., Soleimani-Jelodar, R., Aghadoost, N., Momeni, F., Moridikia, A., Nahand, J.S., Masoudifar, A., Razmjoo, H., Mirzaei, H., 2018. MicroRNAs in retinoblastoma: Potential diagnostic and therapeutic biomarkers. *J Cell Physiol* 233, 3016-3023.
- Gombos, D.S., 2012. Retinoblastoma in the perinatal and neonatal child. *Semin Fetal Neonatal Med* 17, 239-242.

Gombos, D.S., Cauchi, P.A., Hungerford, J.L., Addison, P., Coen, P.G., Kingston, J.E., 2006. Vitreous relapse following primary chemotherapy for retinoblastoma: is adjuvant diode laser a risk factor? *Br J Ophthalmol* 90, 1168-1172.

Gombos, D.S., Hungerford, J., Abramson, D.H., Kingston, J., Chantada, G., Dunkel, I.J., Antoneli, C.B., Greenwald, M., Haik, B.G., Leal, C.A., Medina-Sanson, A., Scheffler, A.C., Veerakul, G., Wieland, R., Bornfeld, N., Wilson, M.W., Yu, C.B., 2007. Secondary acute myelogenous leukemia in patients with retinoblastoma: is chemotherapy a factor? *Ophthalmology* 114, 1378-1383.

Gombos, D.S., Kelly, A., Coen, P.G., Kingston, J.E., Hungerford, J.L., 2002. Retinoblastoma treated with primary chemotherapy alone: the significance of tumour size, location, and age. *Br J Ophthalmol* 86, 80-83.

Gratias, S., Rieder, H., Ullmann, R., Klein-Hitpass, L., Schneider, S., Boloni, R., Kappler, M., Lohmann, D.R., 2007. Allelic loss in a minimal region on chromosome 16q24 is associated with vitreous seeding of retinoblastoma. *Cancer Res* 67, 408-416.

Greenwald, M.J., Strauss, L.C., 1996. Treatment of intraocular retinoblastoma with carboplatin and etoposide chemotherapy. *Ophthalmology* 103, 1989-1997.

Greger, V., Debus, N., Lohmann, D., Hopping, W., Passarge, E., Horsthemke, B., 1994. Frequency and parental origin of hypermethylated RB1 alleles in retinoblastoma. *Hum Genet* 94, 491-496.

Gregersen, P.A., Urbak, S.F., Funding, M., Overgaard, J., Jensen, U.B., Alsner, J., 2016. Danish retinoblastoma patients 1943-2013 - genetic testing and clinical implications. *Acta Oncol* 55, 412-417.

Grobner, S.N., Worst, B.C., Weischenfeldt, J., Buchhalter, I., Kleinheinz, K., Rudneva, V.A., Johann, P.D., Balasubramanian, G.P., Segura-Wang, M., Brabetz, S., Bender, S., Hutter, B., Sturm, D., Pfaff, E., Hubschmann, D., Zipprich, G., Heinold, M., Eils, J., Lawerenz, C., Erkek, S., Lambo, S., Waszak, S., Blattmann, C., Borkhardt, A., Kuhlen, M., Eggert, A., Fulda, S., Gessler, M., Wegert, J., Kappler, R., Baumhoer, D., Burdach, S., Kirschner-Schwabe, R., Kontny, U., Kulozik, A.E., Lohmann, D., Hettmer, S., Eckert, C., Bielack, S., Nathrath, M., Niemeyer, C., Richter, G.H., Schulte, J., Siebert, R., Westermann, F., Molenaar, J.J., Vassal, G., Witt, H., Project, I.P.-S., Project, I.M.-S., Burkhardt, B., Kratz, C.P., Witt, O., van Tilburg, C.M., Kramm, C.M., Fleischhack, G., Dirksen, U., Rutkowski, S., Fruhwald, M., von Hoff, K., Wolf, S., Klingebiel, T., Koscielniak, E., Landgraf, P., Koster, J., Resnick, A.C., Zhang, J., Liu, Y., Zhou, X., Waanders, A.J., Zwijnenburg, D.A., Raman, P., Brors, B., Weber, U.D., Northcott, P.A., Pajtler, K.W., Kool, M., Piro, R.M., Korbel, J.O., Schlesner, M., Eils, R., Jones, D.T.W., Lichter, P., Chavez, L., Zapatka, M., Pfister, S.M., 2018. The landscape of genomic alterations across childhood cancers. *Nature* 555, 321-327.

Grossniklaus, H.E., Dhaliwal, R.S., Martin, D.F., 1998. Diffuse anterior retinoblastoma. *Retina* 18, 238-241.

Group, A.W., Ccm, Group, A.W., 2013. Italian cancer figures, report 2012: Cancer in children and adolescents. *Epidemiol Prev* 37, 1-225.

Gunduz, K., Gunalp, I., Yalcindag, N., Unal, E., Tacyildiz, N., Erden, E., Geyik, P.O., 2004. Causes of chemoreduction failure in retinoblastoma and analysis of associated factors leading to eventual treatment with external beam radiotherapy and enucleation. *Ophthalmology* 111, 1917-1924.

Habib, L.A., Francis, J.H., Fabius, A.W., Gobin, P.Y., Dunkel, I.J., Abramson, D.H., 2018. Second primary malignancies in retinoblastoma patients treated with intra-arterial chemotherapy: the first 10 years. *Br J Ophthalmol* 102, 272-275.

Hadjistilianou, T., De Francesco, S., Martone, G., Malandrini, A., 2006. Retinocytoma associated with calcified vitreous deposits. *Eur J Ophthalmol* 16, 349-351.

Hagstrom, S.A., Dryja, T.P., 1999. Mitotic recombination map of 13cen-13q14 derived from an investigation of loss of heterozygosity in retinoblastomas. *Proc Natl Acad Sci U S A* 96, 2952-2957.

Haider, S., Qureshi, W., Ali, A., 2008. Leukocoria in children. *J Pediatr Ophthalmol Strabismus* 45, 179-180.

Haik, B.G., Dunleavy, S.A., Cooke, C., Ellsworth, R.M., Abramson, D.H., Smith, M.E., Karciglu, Z.A., 1987. Retinoblastoma with anterior chamber extension. *Ophthalmology* 94, 367-370.

Hall, L.S., Ceisler, E., Abramson, D.H., 1999. Visual outcomes in children with bilateral retinoblastoma. *J AAPOS* 3, 138-142.

Halperin, E.C., 2000. Neonatal neoplasms. *Int J Radiat Oncol Biol Phys* 47, 171-178.

Hamama-Raz, Y., Rot, I., Buchbinder, E., 2012. The coping experience of parents of a child with retinoblastoma-malignant eye cancer. *J Psychosoc Oncol* 30, 21-40.

Hamel, P., Heon, E., Gallie, B.L., Budning, A.S., 2000. Focal therapy in the management of retinoblastoma: when to start and when to stop. *J AAPOS* 4, 334-337.

Hartman, R.R., Kompella, U.B., 2018. Intravitreal, Subretinal, and Suprachoroidal Injections: Evolution of Microneedles for Drug Delivery. *J Ocul Pharmacol Ther* 34, 141-153.

Hasanreisoglu, M., Dolz-Marco, R., Ferenczy, S.R., Shields, J.A., Shields, C.L., 2015. Spectral Domain Optical Coherence Tomography Reveals Hidden Fovea Beneath Extensive Vitreous Seeding From Retinoblastoma. *Retina* 35, 1486-1487.

Hayden, B., Jockovich, M.E., Murray, T.G., Kralinger, M.T., Voigt, M., Hernandez, E., Feuer, W., Parel, J.M., 2006. Iontophoretic delivery of carboplatin in a murine model of retinoblastoma. *Invest Ophthalmol Vis Sci* 47, 3717-3721.

Hayden, B.C., Jockovich, M.E., Murray, T.G., Voigt, M., Milne, P., Kralinger, M., Feuer, W.J., Hernandez, E., Parel, J.M., 2004. Pharmacokinetics of systemic versus focal Carboplatin chemotherapy in the rabbit eye: possible implication in the treatment of retinoblastoma. *Invest Ophthalmol Vis Sci* 45, 3644-3649.

Heck, J.E., Lombardi, C.A., Meyers, T.J., Cockburn, M., Wilhelm, M., Ritz, B., 2012. Perinatal characteristics and retinoblastoma. *Cancer Causes Control* 23, 1567-1575.

Herzog, S., Lohmann, D.R., Buiting, K., Schuler, A., Horsthemke, B., Rehder, H., Rieder, H., 2001. Marked differences in unilateral isolated retinoblastomas from young and older children studied by comparative genomic hybridization. *Hum Genet* 108, 98-104.

Hildanus F., 1682. Observationum centuria VI, Observation I. In: *Opera observationem et curationum medico-chiruricarum*. Francofurti : J.L. Dufour., 497-499.

Hilgartner, H., 1903. Report of case of double glioma treated with x-ray. 1903. *Texas Med J*, 322.

Honavar, S.G., Shields, C.L., Shields, J.A., Demirci, H., Naduvilath, T.J., 2001. Intraocular surgery after treatment of retinoblastoma. *Arch Ophthalmol* 119, 1613-1621.

Hosoya, K., Kyoko, H., Toyooka, N., Kato, A., Orihashi, M., Tomi, M., Tachikawa, M., 2008. Evaluation of amino acid-mustard transport as L-type amino acid transporter 1 (LAT1)-mediated alkylating agents. *Biol Pharm Bull* 31, 2126-2130.

Houston, S.K., Lampidis, T.J., Murray, T.G., 2013. Models and discovery strategies for new therapies of retinoblastoma. *Expert Opin Drug Discov* 8, 383-394.

Hudson, L.E., Mendoza, P., Hudson, W.H., Ziesel, A., Hubbard, G.B., 3rd, Wells, J., Dwivedi, B., Kowalski, J., Seby, S., Patel, V., Geisert, E., Specht, C., Grossniklaus, H.E., 2018. Distinct

Gene Expression Profiles Define Anaplastic Grade in Retinoblastoma. *Am J Pathol* 188, 2328-2338.

Hurwitz, M.Y., Marcus, K.T., Chevez-Barrios, P., Louie, K., Aguilar-Cordova, E., Hurwitz, R.L., 1999. Suicide gene therapy for treatment of retinoblastoma in a murine model. *Hum Gene Ther* 10, 441-448.

Hurwitz, R.L., Bogenmann, E., Font, R.L., Holcombe, V., Clark, D., 1990. Expression of the functional cone phototransduction cascade in retinoblastoma. *J Clin Invest* 85, 1872-1878.

Hwang, C.S., Mendoza, P.R., Wells, J.R., Grossniklaus, H.E., Hubbard, G.B., 3rd, 2017. Proliferative Vitreoretinopathy in Treated Retinoblastoma. *Ophthalmol Retina* 1, 165-172.

Imhof, S.M., Moll, A.C., Schouten-van Meeteren, A.Y., 2006. Stage of presentation and visual outcome of patients screened for familial retinoblastoma: nationwide registration in the Netherlands. *Br J Ophthalmol* 90, 875-878.

Inomata, M., Kaneko, A., 1987. Chemosensitivity profiles of primary and cultured human retinoblastoma cells in a human tumor clonogenic assay. *Jpn J Cancer Res* 78, 858-868.

Ishida, Y., Qiu, D., Maeda, M., Fujimoto, J., Kigasawa, H., Kobayashi, R., Sato, M., Okamura, J., Yoshinaga, S., Rikiishi, T., Shichino, H., Kiyotani, C., Kudo, K., Asami, K., Hori, H., Kawaguchi, H., Inada, H., Adachi, S., Manabe, A., Kuroda, T., 2016. Secondary cancers after a childhood cancer diagnosis: a nationwide hospital-based retrospective cohort study in Japan. *Int J Clin Oncol* 21, 506-516.

Jacks, T., Fazeli, A., Schmitt, E.M., Bronson, R.T., Goodell, M.A., Weinberg, R.A., 1992. Effects of an Rb mutation in the mouse. *Nature* 359, 295-300.

Jakobiec, F.A., Tso, M.O., Zimmerman, L.E., Danis, P., 1977. Retinoblastoma and intracranial malignancy. *Cancer* 39, 2048-2058.

Jaradat, I., Yousef, Y.A., Mehyar, M., Sultan, I., Khurma, S., Al-Rawashded, K., Wilson, M., Qaddoumi, I., Salem, A., Alnawaiseh, I., 2011. Retinoblastoma in Jordan: an epidemiological study (2006-2010). *Hematol Oncol Stem Cell Ther* 4, 126-131.

Jehanne, M., Lumbroso-Le Rouic, L., Savignoni, A., Aerts, I., Mercier, G., Bours, D., Desjardins, L., Doz, F., 2009. Analysis of ototoxicity in young children receiving carboplatin in the context of conservative management of unilateral or bilateral retinoblastoma. *Pediatr Blood Cancer* 52, 637-643.

Jenkinson, H., 2015. Retinoblastoma: diagnosis and management--the UK perspective. *Arch Dis Child* 100, 1070-1075.

Ji, X., Hua, P., Li, J., Li, J., Zhao, J., Zhao, P., 2016. Intravitreal Melphalan for Vitreous Seeds: Initial Experience in China. *J Ophthalmol* 2016, 4387286.

Jiang, L.B., Liu, X.Q., Li, B., He, X.J., Jin, Y.L., Li, L.Q., Gao, F., Wang, N.L., 2008. Heat shock proteins and survivin: relationship and effects on proliferation index of retinoblastoma cells. *Histol Histopathol* 23, 827-831.

Jijelava, K.P., Grossniklaus, H.E., 2013. Diffuse anterior retinoblastoma: A review. *Saudi J Ophthalmol* 27, 135-139.

Jubran, R.F., Erdreich-Epstein, A., Butturini, A., Murphree, A.L., Villablanca, J.G., 2004. Approaches to treatment for extraocular retinoblastoma: Children's Hospital Los Angeles experience. *J Pediatr Hematol Oncol* 26, 31-34.

Kaliki, S., Patel, A., Iram, S., Palkonda, V.A.R., 2017. Clinical Presentation and Outcomes of Stage III or Stage IV Retinoblastoma in 80 Asian Indian Patients. *J Pediatr Ophthalmol Strabismus* 54, 177-184.

Kaliki, S., Patel, A., Iram, S., Ramappa, G., Mohamed, A., Palkonda, V.A.R., 2019. RETINOBLASTOMA IN INDIA: Clinical Presentation and Outcome in 1,457 Patients (2,074 Eyes). *Retina*. 39, 379-391.

Kaliki, S., Shields, C.L., Eagle, R.C., Jr., Vemuganti, G.K., Almeida, A., Manjandavida, F.P., Mulay, K., Honavar, S.G., Shields, J.A., 2013a. Ciliary body medulloepithelioma: analysis of 41 cases. *Ophthalmology* 120, 2552-2559.

Kaliki, S., Shields, C.L., Gupta, A., Mishra, D.K., Das, C., Say, E.A., Shields, J.A., 2015a. Newly Diagnosed Active Retinoblastoma in Adults. *Retina* 35, 2483-2488.

Kaliki, S., Shields, C.L., Rojanaporn, D., Al-Dahmash, S., McLaughlin, J.P., Shields, J.A., Eagle, R.C., Jr., 2013b. High-risk retinoblastoma based on international classification of retinoblastoma: analysis of 519 enucleated eyes. *Ophthalmology* 120, 997-1003.

Kaliki, S., Srinivasan, V., Gupta, A., Mishra, D.K., Naik, M.N., 2015b. Clinical features predictive of high-risk retinoblastoma in 403 Asian Indian patients: a case-control study. *Ophthalmology* 122, 1165-1172.

Kaliki, S., Taneja, S., Palkonda, V.A.R., 2018. INADVERTENT INTRAOCULAR SURGERY IN CHILDREN WITH UNSUSPECTED RETINOBLASTOMA: A Study of 14 Cases. *Retina*.

Kalita, D., Shome, D., Jain, V.G., Chadha, K., Bellare, J.R., 2014. In vivo intraocular distribution and safety of periocular nanoparticle carboplatin for treatment of advanced retinoblastoma in humans. *Am J Ophthalmol* 157, 1109-1115.

Kanber, D., Berulava, T., Ammerpohl, O., Mitter, D., Richter, J., Siebert, R., Horsthemke, B., Lohmann, D., Buiting, K., 2009. The human retinoblastoma gene is imprinted. *PLoS Genet* 5, e1000790.

Kaneko, A., Suzuki, S., 2003. Eye-preservation treatment of retinoblastoma with vitreous seeding. *Jpn J Clin Oncol* 33, 601-607.

Kaneko, A., Takayama J et al, 1990. Chemo-thermotherapy was successful in two cases of recurrence of intraocular retinoblastoma after irradiation. *Rinsho Ganka (Jpn J Clin Ophthalmol)* 44, 289-292.

Kao, L.Y., 2000. Diffuse infiltrating retinoblastoma: an inherited case. *Retina* 20, 217-219.

Kapatai, G., Brundler, M.A., Jenkinson, H., Kearns, P., Parulekar, M., Peet, A.C., McConville, C.M., 2013. Gene expression profiling identifies different sub-types of retinoblastoma. *Br J Cancer* 109, 512-525.

Karatza, E.C., Shields, C.L., Flanders, A.E., Gonzalez, M.E., Shields, J.A., 2006. Pineal cyst simulating pinealoblastoma in 11 children with retinoblastoma. *Arch Ophthalmol* 124, 595-597.

Karcioglu, Z.A., al-Mesfer, S.A., Abboud, E., Jabak, M.H., Mullaney, P.B., 1997. Workup for metastatic retinoblastoma. A review of 261 patients. *Ophthalmology* 104, 307-312.

Kase, S., Parikh, J.G., Rao, N.A., 2008. Peripapillary subretinal neovascularization in retinoblastoma. *Graefes Arch Clin Exp Ophthalmol* 246, 931-934.

Kaufmann, S.H., Peereboom, D., Buckwalter, C.A., Svingen, P.A., Grochow, L.B., Donehower, R.C., Rowinsky, E.K., 1996. Cytotoxic effects of topotecan combined with various anticancer agents in human cancer cell lines. *J Natl Cancer Inst* 88, 734-741.

Kelly, A., Kaufman, S.C., Ali, R., Grajewski, A., Anderson, J., 2016. Rare Anterior Segment Retinoblastoma Masquerading as Corneal Endotheliitis. *Eye Contact Lens* 42, e1-3.

Khandekar, R., Ganesh, A., Al Lawati, J., 2004. A 12-year epidemiological review of retinoblastoma in Omani children. *Ophthalmic Epidemiol* 11, 151-159.

- Kim, H., Lee, J.W., Kang, H.J., Park, H.J., Kim, Y.Y., Shin, H.Y., Yu, Y.S., Kim, I.H., Ahn, H.S., 2008. Clinical results of chemotherapy based treatment in retinoblastoma patients: a single center experience. *Cancer Res Treat* 40, 164-171.
- Kim, J.M., Kim, J.H., Kim, S.J., Park, K.D., Shin, H.Y., Ahn, H.S., Yu, Y.S., 2010a. Visual prognosis of retinoblastoma in the posterior pole treated with primary chemotherapy plus local treatments. *Korean J Ophthalmol* 24, 347-352.
- Kim, J.W., Ngai, L.K., Sadda, S., Murakami, Y., Lee, D.K., Murphree, A.L., 2014. Retcam fluorescein angiography findings in eyes with advanced retinoblastoma. *Br J Ophthalmol* 98, 1666-1671.
- Kim, J.W., Yau, J.W., Moshfeghi, D., Fishman, M., 2010b. Orbital fibrosis and intraocular recurrence of retinoblastoma following periocular carboplatin. *J Pediatr Ophthalmol Strabismus* 47 Online, e1-4.
- King, B.A., Parra, C., Li, Y., Helton, K.J., Qaddoumi, I., Wilson, M.W., Ogg, R.J., 2015. Spatiotemporal Patterns of Tumor Occurrence in Children with Intraocular Retinoblastoma. *PLoS One* 10, e0132932.
- Kingston, J.E., Hungerford, J.L., Madreperla, S.A., Plowman, P.N., 1996. Results of combined chemotherapy and radiotherapy for advanced intraocular retinoblastoma. *Arch Ophthalmol* 114, 1339-1343.
- Kiratli, H., Koc, I., Varan, A., Akyuz, C., 2017. Intravitreal chemotherapy in the management of vitreous disease in retinoblastoma. *Eur J Ophthalmol* 27, 423-427.
- Kivela, T., Polkunen, M.L., 2003. Pieter Pauw's tumor oculorum: reappraisal of the presumed first description of retinoblastoma in 1597. *Arch Ophthalmol* 121, 881-886.
- Kivela, T., 1999. Trilateral retinoblastoma: a meta-analysis of hereditary retinoblastoma associated with primary ectopic intracranial retinoblastoma. *J Clin Oncol* 17, 1829-1837.
- Kivela, T., 2009. The epidemiological challenge of the most frequent eye cancer: retinoblastoma, an issue of birth and death. *Br J Ophthalmol* 93, 1129-1131.
- Kivela, T.T., Hadjistilianou, T., 2017. Neonatal Retinoblastoma. *Asia Pac J Oncol Nurs* 4, 197-204.
- Kleinerman, R.A., Tucker, M.A., Tarone, R.E., Abramson, D.H., Seddon, J.M., Stovall, M., Li, F.P., Fraumeni, J.F., Jr., 2005. Risk of new cancers after radiotherapy in long-term survivors of retinoblastoma: an extended follow-up. *J Clin Oncol* 23, 2272-2279.

Kleinerman, R.A., Yu, C.L., Little, M.P., Li, Y., Abramson, D., Seddon, J., Tucker, M.A., 2012. Variation of second cancer risk by family history of retinoblastoma among long-term survivors. *J Clin Oncol* 30, 950-957.

Klufas, M.A., Gobin, Y.P., Marr, B., Brodie, S.E., Dunkel, I.J., Abramson, D.H., 2012. Intra-arterial chemotherapy as a treatment for intraocular retinoblastoma: alternatives to direct ophthalmic artery catheterization. *AJNR Am J Neuroradiol* 33, 1608-1614.

Klutznick, M., Brockmann, D., Lohmann, D.R., 2002. A parent-of-origin effect in two families with retinoblastoma is associated with a distinct splice mutation in the RB1 gene. *Am J Hum Genet* 71, 174-179.

Knudson, A.G., Jr., 1971. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci U S A* 68, 820-823.

Koelbing, M.H., 1954. Fabricius Hildanus' understanding and treatment of intra-ocular tumors. *Ophthalmologica* 127, 288-93.

Kohe, S., Brundler, M.A., Jenkinson, H., Parulekar, M., Wilson, M., Peet, A.C., McConville, C.M., Children's, C., Leukaemia, G., 2015. Metabolite profiling in retinoblastoma identifies novel clinicopathological subgroups. *Br J Cancer* 113, 1216-1224.

Kooi, I.E., Mol, B.M., Massink, M.P., Ameziame, N., Meijers-Heijboer, H., Dommering, C.J., van Mil, S.E., de Vries, Y., van der Hout, A.H., Kaspers, G.J., Moll, A.C., Te Riele, H., Cloos, J., Dorsman, J.C., 2016a. Somatic genomic alterations in retinoblastoma beyond RB1 are rare and limited to copy number changes. *Sci Rep* 6, 25264.

Kooi, I.E., Mol, B.M., Massink, M.P., de Jong, M.C., de Graaf, P., van der Valk, P., Meijers-Heijboer, H., Kaspers, G.J., Moll, A.C., Te Riele, H., Cloos, J., Dorsman, J.C., 2016b. A Meta-Analysis of Retinoblastoma Copy Numbers Refines the List of Possible Driver Genes Involved in Tumor Progression. *PLoS One* 11, e0153323.

Kooi, I.E., Mol, B.M., Moll, A.C., van der Valk, P., de Jong, M.C., de Graaf, P., van Mil, S.E., Schouten-van Meeteren, A.Y., Meijers-Heijboer, H., Kaspers, G.L., Te Riele, H., Cloos, J., Dorsman, J.C., 2015. Loss of photoreceptor and gain of genomic alterations in retinoblastoma reveal tumor progression. *EBioMedicine* 2, 660-670.

Kramer, K., Kushner, B.H., Modak, S., Pandit-Taskar, N., Smith-Jones, P., Zanzonico, P., Humm, J.L., Xu, H., Wolden, S.L., Souweidane, M.M., Larson, S.M., Cheung, N.K., 2010. Compartmental intrathecal radioimmunotherapy: results for treatment for metastatic CNS neuroblastoma. *J Neurooncol* 97, 409-418.

Kroll, M.E., Carpenter, L.M., Murphy, M.F., Stiller, C.A., 2012. Effects of changes in diagnosis and registration on time trends in recorded childhood cancer incidence in Great Britain. *Br J Cancer* 107, 1159-1162.

Kunkele, A., Jurklies, C., Wieland, R., Lohmann, D., Bornfeld, N., Eggert, A., Schulte, J.H., 2013. Chemoreduction improves eye retention in patients with retinoblastoma: a report from the German Retinoblastoma Reference Centre. *Br J Ophthalmol* 97, 1277-1283.

Kunkele, A., Wilm, J., Holdt, M., Lohmann, D., Bornfeld, N., Eggert, A., Temming, P., Schulte, J.H., 2015. Neoadjuvant/adjuvant treatment of high-risk retinoblastoma: a report from the German Retinoblastoma Referral Centre. *Br J Ophthalmol* 99, 949-953.

Laurent, V.E., Torbidoni, A.V., Sampor, C., Ottaviani, D., Vazquez, V., Gabri, M.R., Garcia de Davila, M.T., Ramirez-Ortiz, M.A., Alonso, C.N., Rossi, J., Alonso D.F., Chantada, G.L., 2016. Minimal disseminated disease in nonmetastatic retinoblastoma with high-risk pathologic features and association with disease-free survival. *JAMA Ophthalmol* 134, 1374-1379.

Laurent, V.E., Sampor, C., Solernou, V., Rossi, J., Gabri, M., Eandi-Eberle, S., de Davila, M.T., Alonso, D.F., Chantada, G.L., 2013. Detection of minimally disseminated disease in the cerebrospinal fluid of children with high-risk retinoblastoma by reverse transcriptase-polymerase chain reaction for GD2 synthase mRNA. *Eur J Cancer* 49, 2892-2899.

Laurie, N.A., Gray, J.K., Zhang, J., Leggas, M., Relling, M., Egorin, M., Stewart, C., Dyer, M.A., 2005. Topotecan combination chemotherapy in two new rodent models of retinoblastoma. *Clin Cancer Res* 11, 7569-7578.

Le Goff, M.M., Bishop, P.N., 2008. Adult vitreous structure and postnatal changes. *Eye (Lond)* 22, 1214-1222.

Leal-Leal, C.A., Rivera-Luna, R., Flores-Rojas, M., Juarez-Echenique, J.C., Ordaz, J.C., Amador-Zarco, J., 2006. Survival in extra-orbital metastatic retinoblastoma: treatment results. *Clin Transl Oncol* 8, 39-44.

Leander, C., Fu, L.C., Pena, A., Howard, S.C., Rodriguez-Galindo, C., Wilimas, J.A., Ribeiro, R.C., Haik, B., 2007. Impact of an education program on late diagnosis of retinoblastoma in Honduras. *Pediatr Blood Cancer* 49, 817-819.

Lee, E.Y., Bookstein, R., Young, L.J., Lin, C.J., Rosenfeld, M.G., Lee, W.H., 1988. Molecular mechanism of retinoblastoma gene inactivation in retinoblastoma cell line Y79. *Proc Natl Acad Sci U S A* 85, 6017-6021.

Lee, E.Y., Chang, C.Y., Hu, N., Wang, Y.C., Lai, C.C., Herrup, K., Lee, W.H., Bradley, A., 1992. Mice deficient for Rb are nonviable and show defects in neurogenesis and haematopoiesis. *Nature* 359, 288-294.

Lee, T.C., Almeida, D., Claros, N., Abramson, D.H., Cobrinik, D., 2006. Cell cycle-specific and cell type-specific expression of Rb in the developing human retina. *Invest Ophthalmol Vis Sci* 47, 5590-5598.

Lee, W.H., Bookstein, R., Hong, F., Young, L.J., Shew, J.Y., Lee, E.Y., 1987. Human retinoblastoma susceptibility gene: cloning, identification, and sequence. *Science* 235, 1394-1399.

Li, S.Y., Chen, S.C., Tsai, C.F., Sheu, S.M., Yeh, J.J., Tsai, C.B., 2016. Incidence and survival of retinoblastoma in Taiwan: a nationwide population-based study 1998-2011. *Br J Ophthalmol* 100, 839-842.

Lim, T.H., Robertson, D.M., 2000. Presumed rhegmatogenous retinal detachment in patients with retinoblastoma. *Retina* 20, 22-27.

Lima, N.N., do Nascimento, V.B., de Carvalho, S.M., Neto, M.L., Moreira, M.M., Brasil, A.Q., Junior, F.T., de Oliveira, G.F., Reis, A.O., 2013. Spirituality in childhood cancer care. *Neuropsychiatr Dis Treat* 9, 1539-1544.

Linn Murphree, A., 2005. Intraocular retinoblastoma: the case for a new group classification. *Ophthalmol Clin North Am* 18, 41-53, viii.

Lohmann, D.R., Gallie, B.L., 2004. Retinoblastoma: revisiting the model prototype of inherited cancer. *Am J Med Genet C Semin Med Genet* 129C, 23-28.

Lu, J.E., Francis, J.H., Dunkel, I.J., Shields, C.L., Yu, M.D., Berry, J.L., Kogachi, K., Skalet, A.H., Miller, A.K., Santapuram, P.R., Daniels, A.B., Abramson, D.H., 2018. Metastases and death rates after primary enucleation of unilateral retinoblastoma in the USA 2007-2017. *Br J Ophthalmol*.

Lueder, G.T., Heon, E., Gallie, B.L., 1995. Retinoma associated with vitreous seeding. *Am J Ophthalmol* 119, 522-523.

Lumbroso-Le Rouic, L., Aerts, I., Levy-Gabriel, C., Dendale, R., Sastre, X., Esteve, M., Asselain, B., Bours, D., Doz, F., Desjardins, L., 2008. Conservative treatments of intraocular retinoblastoma. *Ophthalmology* 115, 1405-1410, 1410 e1401-1402.

Lumbroso, L., Doz, F., Urbiet, M., Levy, C., Bours, D., Asselain, B., Vedrenne, J., Zucker, J.M., Desjardins, L., 2002. Chemothermotherapy in the management of retinoblastoma. *Ophthalmology* 109, 1130-1136.

Luna-Fineman, S., S., F.-A., A., A., G., A., M., B., R., B., ME., C., J., G., V., G., MA., G., L., F., C., M., G., M., RA., M., R., O., Chantada, G., Rodriguez-Galindo, C., 2018. Delayed enucleation with neoadjuvant chemotherapy in advanced intraocular unilateral retinoblastoma: AHOPCA-II, a prospective, multi-instituional protocol in Central America. *J Clin Oncol* in press.

Ma, B., Lei, X., Guan, Y., Mou, L.S., Yuan, Y.F., Yue, H., Lu, Y., Xu, G.T., Qian, J., 2011. Maintenance of retinal cancer stem cell-like properties through long-term serum-free culture from human retinoblastoma. *Oncol Rep* 26, 135-143.

Maat-Kievit, J.A., Oepkes, D., Hartwig, N.G., Vermeij-Keers, C., van Kamp, I.L., van de Kamp, J.J., 1993. A large retinoblastoma detected in a fetus at 21 weeks of gestation. *Prenat Diagn* 13, 377-384.

MacCarthy, A., Birch, J.M., Draper, G.J., Hungerford, J.L., Kingston, J.E., Kroll, M.E., Onadim, Z., Stiller, C.A., Vincent, T.J., Murphy, M.F., 2009. Retinoblastoma in Great Britain 1963-2002. *Br J Ophthalmol* 93, 33-37.

MacCarthy, A., Draper, G.J., Steliarova-Foucher, E., Kingston, J.E., 2006. Retinoblastoma incidence and survival in European children (1978-1997). Report from the Automated Childhood Cancer Information System project. *Eur J Cancer* 42, 2092-2102.

MacKay, C.J., Abramson, D.H., Ellsworth, R.M., 1984. Metastatic patterns of retinoblastoma. *Arch Ophthalmol* 102, 391-396.

Macpherson, D., 2008. Insights from mouse models into human retinoblastoma. *Cell Div* 3, 9.

MacPherson, D., Conkrite, K., Tam, M., Mukai, S., Mu, D., Jacks, T., 2007. Murine bilateral retinoblastoma exhibiting rapid-onset, metastatic progression and N-myc gene amplification. *EMBO J* 26, 784-794.

Madreperla, S.A., Hungerford, J.L., Cooling, R.J., Sullivan, P., Gregor, Z., 2000. Repair of late retinal detachment after successful treatment of retinoblastoma. *Retina* 20, 28-32.

Maidana, D.E., Pellegrini, M., Shields, J.A., Shields, C.L., 2014. Choroidal thickness after intraarterial chemotherapy for retinoblastoma. *Retina* 34, 2103-2109.

- Mallam, J.N., Hurwitz, M.Y., Mahoney, T., Chevez-Barrios, P., Hurwitz, R.L., 2004. Efficient gene transfer into retinal cells using adenoviral vectors: dependence on receptor expression. *Invest Ophthalmol Vis Sci* 45, 1680-1687.
- Mallipatna, A., Gallie, B., Chévez-Barrios, P., Lumbroso-Le Rouic, L., Chantada, G., Doz, F., Brisse, H., Munier, F., Albert, D., Catala-Mora, J., 2017. Retinoblastoma. *AJCC cancer staging manual* 8, 819-831.
- Mallipatna, A.C., Dimaras, H., Chan, H.S., Heon, E., Gallie, B.L., 2011. Periocular topotecan for intraocular retinoblastoma. *Arch Ophthalmol* 129, 738-745.
- Manjandavida, F.P., Honavar, S.G., Reddy, V.A., Khanna, R., 2014. Management and outcome of retinoblastoma with vitreous seeds. *Ophthalmology* 121, 517-524.
- Marees, T., Moll, A.C., Imhof, S.M., de Boer, M.R., Ringens, P.J., van Leeuwen, F.E., 2008. Risk of second malignancies in survivors of retinoblastoma: more than 40 years of follow-up. *J Natl Cancer Inst* 100, 1771-1779.
- Marees, T., van Leeuwen, F.E., de Boer, M.R., Imhof, S.M., Ringens, P.J., Moll, A.C., 2009. Cancer mortality in long-term survivors of retinoblastoma. *Eur J Cancer* 45, 3245-3253.
- Marees, T., van Leeuwen, F.E., Schaapveld, M., Imhof, S.M., de Boer, M.R., Kors, W.A., Ringens, P.J., Moll, A.C., 2010. Risk of third malignancies and death after a second malignancy in retinoblastoma survivors. *Eur J Cancer* 46, 2052-2058.
- Margo, C., Hidayat, A., Kopelman, J., Zimmerman, L.E., 1983. Retinocytoma. A benign variant of retinoblastoma. *Arch Ophthalmol* 101, 1519-1531.
- Marr, B.P., Dunkel, I.J., Linker, A., Abramson, D.H., 2012. Periocular carboplatin for retinoblastoma: long-term report (12 years) on efficacy and toxicity. *Br J Ophthalmol* 96, 881-883.
- Mataftsi, A., Zografos, L., Balmer, A., Uffer, S., Stupp, R., Janzer, R.C., Pica, A., Schorderet, D.F., Munier, F.L., 2012. Chiasmatic infiltration secondary to late malignant transformation of retinoma. *Ophthalmic Genet* 33, 155-158.
- McEvoy, J., Flores-Otero, J., Zhang, J., Nemeth, K., Brennan, R., Bradley, C., Krafcik, F., Rodriguez-Galindo, C., Wilson, M., Xiong, S., Lozano, G., Sage, J., Fu, L., Louhibi, L., Trimarchi, J., Pani, A., Smeyne, R., Johnson, D., Dyer, M.A., 2011. Coexpression of normally incompatible developmental pathways in retinoblastoma genesis. *Cancer Cell* 20, 260-275.

- McEvoy, J., Nagahawatte, P., Finkelstein, D., Richards-Yutz, J., Valentine, M., Ma, J., Mullighan, C., Song, G., Chen, X., Wilson, M., Brennan, R., Pounds, S., Becksfort, J., Huether, R., Lu, C., Fulton, R.S., Fulton, L.L., Hong, X., Dooling, D.J., Ochoa, K., Mardis, E.R., Wilson, R.K., Easton, J., Zhang, J., Downing, J.R., Ganguly, A., Dyer, M.A., 2014. RB1 gene inactivation by chromothripsis in human retinoblastoma. *Oncotarget* 5, 438-450.
- McFall, R.C., Sery, T.W., Makadon, M., 1977. Characterization of a new continuous cell line derived from a human retinoblastoma. *Cancer Res* 37, 1003-1010.
- Meier, P., Sterker, I., Tegetmeyer, H., 2006. [Leucocoria in childhood]. *Klin Monbl Augenheilkd* 223, 521-527.
- Mendelsohn, M.E., Abramson, D.H., Madden, T., Tong, W., Tran, H.T., Dunkel, I.J., 1998. Intraocular concentrations of chemotherapeutic agents after systemic or local administration. *Arch Ophthalmol* 116, 1209-1212.
- Mendoza, P.R., Specht, C.S., Hubbard, G.B., Wells, J.R., Lynn, M.J., Zhang, Q., Kong, J., Grossniklaus, H.E., 2015. Histopathologic grading of anaplasia in retinoblastoma. *Am J Ophthalmol* 159, 764-776.
- Metz, C.H., Bornfeld, N., Metz, K.A., Gok, M., 2016. Suspected vitreous seeding of uveal melanoma: relevance of diagnostic vitrectomy. *Br J Ophthalmol* 100, 660-664.
- Meyer, C.H., Michels, S., Rodrigues, E.B., Hager, A., Mennel, S., Schmidt, J.C., Helb, H.M., Farah, M.E., 2011. Incidence of rhegmatogenous retinal detachments after intravitreal antivascular endothelial factor injections. *Acta Ophthalmol* 89, 70-75.
- Miller, D.M., Murray, T.G., Cicciarelli, N.L., Capo, H., Markoe, A.M., 2005. Pars plana lensectomy and intraocular lens implantation in pediatric radiation-induced cataracts in retinoblastoma. *Ophthalmology* 112, 1620-1624.
- Mitter, D., Ullmann, R., Muradyan, A., Klein-Hitpass, L., Kanber, D., Ounap, K., Kaulisch, M., Lohmann, D., 2011. Genotype-phenotype correlations in patients with retinoblastoma and interstitial 13q deletions. *Eur J Hum Genet* 19, 947-958.
- Mohney, B.G., Elner, V.M., Smith, A.B., Harbour, J.W., Smith, B.D., Musch, D.C., Smith, S.J., 2017. Preclinical Acute Ocular Safety Study of Combined Intravitreal Carboplatin and Etoposide Phosphate for Retinoblastoma. *Ophthalmic Surg Lasers Imaging Retina* 48, 151-159.
- Moll, A.C., Dijk, J.v., Bosscha, M.I., Dommering, C.J., Graaf, P.d., Kors, W.A., Leeuwen, F.E.v., 2013. Second malignancies and other long-term effects in retinoblastoma survivors, *Retinoblastoma: Clinical Advances and Emerging Treatment Strategies*, pp. 110-124.

Moll, A.C., Dommering, C.J., Bosscha, M.I., de Graaf, P., Kors, W.A., van Leeuwen, F.E., 2012. Risk factors for the incidence of second cancers in survivors of retinoblastoma with a family history. *J Clin Oncol* 30, 3028; author reply 3028-3029.

Moll, A.C., Imhof, S.M., Bouter, L.M., Tan, K.E., 1997. Second primary tumors in patients with retinoblastoma. A review of the literature. *Ophthalmic Genet* 18, 27-34.

Moll, A.C., Imhof, S.M., Kuik, D.J., Bouter, L.M., Den Otter, W., Bezemer, P.D., Kote, J.W., Tan, K.E., 1996. High parental age is associated with sporadic hereditary retinoblastoma: the Dutch retinoblastoma register 1862-1994. *Hum Genet* 98, 109-112.

Moll, A.C., Imhof, S.M., Schouten-Van Meeteren, A.Y., Boers, M., 2002. Screening for pineoblastoma in patients with retinoblastoma. *Arch Ophthalmol* 120, 1774; author reply 1774.

Moll, A.C., Imhof, S.M., Schouten-Van Meeteren, A.Y., Kuik, D.J., Hofman, P., Boers, M., 2001. Second primary tumors in hereditary retinoblastoma: a register-based study, 1945-1997: is there an age effect on radiation-related risk? *Ophthalmology* 108, 1109-1114.

Moreno, F., Sinaki, B., Fandino, A., Dussel, V., Orellana, L., Chantada, G., 2014. A population-based study of retinoblastoma incidence and survival in Argentine children. *Pediatr Blood Cancer* 61, 1610-1615.

Moscinski, L.C., Pendergrass, T.W., Weiss, A., Hvizdala, E., Buckley, K.S., Kalina, R.E., 1996. Recommendations for the use of routine bone marrow aspiration and lumbar punctures in the follow-up of patients with retinoblastoma. *J Pediatr Hematol Oncol* 18, 130-134.

Motegi, T., Kaga, M., Yanagawa, Y., Kadowaki, H., Watanabe, K., Inoue, A., Komatsu, M., Minoda, K., 1983. A recognizable pattern of the midface of retinoblastoma patients with interstitial deletion of 13q. *Hum Genet* 64, 160-162.

Moulin, A.P., Gaillard, M.C., Balmer, A., Munier, F.L., 2012. Ultrasound biomicroscopy evaluation of anterior extension in retinoblastoma: a clinicopathological study. *Br J Ophthalmol* 96, 337-340.

Muen, W.J., Kingston, J.E., Robertson, F., Brew, S., Sagoo, M.S., Reddy, M.A., 2012. Efficacy and complications of super-selective intra-ophthalmic artery melphalan for the treatment of refractory retinoblastoma. *Ophthalmology* 119, 611-616.

Mullaney, P.B., Abboud, E.B., Al-Mesfer, S.A., 1997. Retinal detachment associated with type III retinoblastoma regression after cryotherapy and external-beam radiotherapy. *Am J Ophthalmol* 123, 140-142.

- Mulvihill, A., Budning, A., Jay, V., Vandenhoven, C., Heon, E., Gallie, B.L., Chan, H.S., 2003. Ocular motility changes after subtenon carboplatin chemotherapy for retinoblastoma. *Arch Ophthalmol* 121, 1120-1124.
- Munier, F., Pescia, G., Jotterand-Bellomo, M., Balmer, A., Gailloud, C., Thonney, F., 1989. Constitutional karyotype in retinoblastoma. Case report and review of literature. *Ophthalmic Paediatr Genet* 10, 129-150.
- Munier, F.L., 2014. Classification and management of seeds in retinoblastoma. Ellsworth Lecture Ghent August 24th 2013. *Ophthalmic Genet* 35, 193-207.
- Munier, F.L., Balmer, A., van Melle, G., Gailloud, C., 1994. Radial asymmetry in the topography of retinoblastoma. Clues to the cell of origin. *Ophthalmic Genet* 15, 101-106.
- Munier, F.L., Beck-Popovic, M., Balmer, A., Gaillard, M.C., Bovey, E., Binaghi, S., 2011. Occurrence of sectoral choroidal occlusive vasculopathy and retinal arteriolar embolization after superselective ophthalmic artery chemotherapy for advanced intraocular retinoblastoma. *Retina* 31, 566-573.
- Munier, F.L., Gaillard, M.-C., Decembrini, S., Beck-Popovic, M., 2015. Aqueous seeding: fall of the ultimate intraocular retinoblastoma sanctuary by a new in situ chemotherapy technique. *Investigative Ophthalmology & Visual Science* 56, 1663-1663.
- Munier, F.L., Gaillard, M.C., Balmer, A., Soliman, S., Podilsky, G., Moulin, A.P., Beck-Popovic, M., 2012a. Intravitreal chemotherapy for vitreous disease in retinoblastoma revisited: from prohibition to conditional indications. *Br J Ophthalmol* 96, 1078-1083.
- Munier, F.L., Gaillard, M.C., Decembrini, S., Bongiovanni, M., Beck-Popovic, M., 2017a. Intracameral Chemotherapy (Melfalan) for Aqueous Seeding in Retinoblastoma: Bicameral Injection Technique and Related Toxicity in a Pilot Case Study. *Ocul Oncol Pathol* 3, 149-155.
- Munier, F.L., Mosimann, P., Puccinelli, F., Gaillard, M.C., Stathopoulos, C., Houghton, S., Bergin, C., Beck-Popovic, M., 2017b. First-line intra-arterial versus intravenous chemotherapy in unilateral sporadic group D retinoblastoma: evidence of better visual outcomes, ocular survival and shorter time to success with intra-arterial delivery from retrospective review of 20 years of treatment. *Br J Ophthalmol* 101, 1086-1093.
- Munier, F.L., Moulin, A., Gaillard, M.C., Bongiovanni, M., Decembrini, S., Houghton, S., Beck-Popovic, M., Stathopoulos, C., 2018. Intracameral Chemotherapy for Globe Salvage in Retinoblastoma with Secondary Anterior Chamber Invasion. *Ophthalmology* 125, 615-617.

Munier, F.L., Soliman, S., Moulin, A.P., Gaillard, M.C., Balmer, A., Beck-Popovic, M., 2012b. Profiling safety of intravitreal injections for retinoblastoma using an anti-reflux procedure and sterilisation of the needle track. *Br J Ophthalmol* 96, 1084-1087.

Munier, F.L., Verwey, J., Pica, A., Balmer, A., Zografos, L., Abouzeid, H., Timmerman, B., Goitein, G., Moeckli, R., 2008. New developments in external beam radiotherapy for retinoblastoma: from lens to normal tissue-sparing techniques. *Clin Exp Ophthalmol* 36, 78-89.

Munier, F.L., Wang, M.X., Spence, M.A., Thonney, F., Balmer, A., Pescia, G., Donoso, L.A., Murphree, A.L., 1993. Pseudo low penetrance in retinoblastoma. Fortuitous familial aggregation of sporadic cases caused by independently derived mutations in two large pedigrees. *Arch Ophthalmol* 111, 1507-1511.

Murakami, N., Suzuki, S., Ito, Y., Yoshimura, R., Inaba, K., Kuroda, Y., Morota, M., Mayahara, H., Sakudo, M., Wakita, A., Okamoto, H., Sumi, M., Kagami, Y., Nakagawa, K., Ohtomo, K., Itami, J., 2012. (1)(0)(6)Ruthenium plaque therapy (RPT) for retinoblastoma. *Int J Radiat Oncol Biol Phys* 84, 59-65.

Murphree, A.L., Villablanca, J.G., Deegan, W.F., 3rd, Sato, J.K., Malogolowkin, M., Fisher, A., Parker, R., Reed, E., Gomer, C.J., 1996. Chemotherapy plus local treatment in the management of intraocular retinoblastoma. *Arch Ophthalmol* 114, 1348-1356.

Narang, S., Mashayekhi, A., Rudich, D., Shields, C.L., 2012. Predictors of long-term visual outcome after chemoreduction for management of intraocular retinoblastoma. *Clin Exp Ophthalmol* 40, 736-742.

Naru, J., Aggarwal, R., Mohanty, A.K., Singh, U., Bansal, D., Kakkar, N., Agnihotri, N., 2017. Identification of differentially expressed proteins in retinoblastoma tumors using mass spectrometry-based comparative proteomic approach. *J Proteomics* 159, 77-91.

Nawaiseh, I., Al-Hussaini, M., Alhamwi, A., Meyar, M., Sultan, I., Alrawashdeh, K., Jaradat, I., Yousef, Y.A., 2015. The impact of growth patterns of retinoblastoma (endophytic, exophytic, and mixed patterns). *Turk Patoloji Derg* 31, 45-50.

Nemeth, K.M., Federico, S., Carcaboso, A.M., Shen, Y., Schaiquevich, P., Zhang, J., Egorin, M., Stewart, C., Dyer, M.A., 2011. Subconjunctival carboplatin and systemic topotecan treatment in preclinical models of retinoblastoma. *Cancer* 117, 421-434.

Ohnishi, Y., Yamana, Y., Minei, M., Ibayashi, H., 1982. Application of fluorescein angiography in retinoblastoma. *Am J Ophthalmol* 93, 578-588.

- Ong, S.J., Chao, A.N., Wong, H.F., Liou, K.L., Kao, L.Y., 2015. Selective ophthalmic arterial injection of melphalan for intraocular retinoblastoma: a 4-year review. *Jpn J Ophthalmol* 59, 109-117.
- Osman, I.M., Abouzeid, H., Balmer, A., Gaillard, M.C., Othenin-Girard, P., Pica, A., Moeckli, R., Schorderet, D.F., Munier, F.L., 2011. Modern cataract surgery for radiation-induced cataracts in retinoblastoma. *Br J Ophthalmol* 95, 227-230.
- Paez-Escamilla, M., Bagheri, N., Teira, L.E., Corrales-Medina, F.F., Harbour, W., 2017. Intracameral Topotecan Hydrochloride for Anterior Chamber Seeding of Retinoblastoma. *JAMA Ophthalmol* 135, 1453-1454.
- Palamar, M., Pirondini, C., Shields, C.L., Shields, J.A., 2008. Cavitary retinoblastoma: ultrasonographic and fluorescein angiographic findings in 3 cases. *Arch Ophthalmol* 126, 1598-1600.
- Palazzi, M., Abramson, D.H., Ellsworth, R.M., 1990. Endophytic vs exophytic unilateral retinoblastoma: is there any real difference? *J Pediatr Ophthalmol Strabismus* 27, 255-258.
- Palioura, S., Gobin, Y.P., Brodie, S.E., Marr, B.P., Dunkel, I.J., Abramson, D.H., 2012. Ophthalmic artery chemosurgery for the management of retinoblastoma in eyes with extensive (>50%) retinal detachment. *Pediatr Blood Cancer* 59, 859-864.
- Palma, J., Sasso, D.F., Dufort, G., Koop, K., Sampor, C., Diez, B., Richard, L., Castillo, L., Chantada, G.L., 2012. Successful treatment of metastatic retinoblastoma with high-dose chemotherapy and autologous stem cell rescue in South America. *Bone Marrow Transplant* 47, 522-527.
- Pant, G., Verma, N., Kumar, A., Pooniya, V., Gupta, S.K., 2017. Outcome of extraocular retinoblastoma in a resource limited center from low middle income country. *Pediatr Hematol Oncol* 34, 419-424.
- Paquette, L.B., Miller, D., Jackson, H.A., Lee, T., Randolph, L., Murphree, A.L., Panigrahy, A., 2012. In utero detection of retinoblastoma with fetal magnetic resonance and ultrasound: initial experience. *AJP reports* 2, 55.
- Park, S.J., Woo, S.J., Park, K.H., 2014. Incidence of retinoblastoma and survival rate of retinoblastoma patients in Korea using the Korean National Cancer Registry database (1993-2010). *Invest Ophthalmol Vis Sci* 55, 2816-2821.

Parness-Yossifon, R., Bryar, P.J., Weinstein, J.L., Srikumaran, D., Mets, M.B., 2009. Sudden dispersion of retinoblastoma shortly after initial chemotherapy treatment. *Am J Ophthalmol* 147, 903-906.

Pascual-Pasto, G., Bazan-Peregrino, M., Olaciregui, N.G., Restrepo-Perdomo, C.A., Mato-Berciano, A., Ottaviani, D., Weber, K., Correa, G., Paco, S., Vila-Ubach, M., Cuadrado-Vilanova, M., Castillo-Ecija, H., Botteri, G., Garcia-Gerique, L., Moreno-Gilabert, H., Gimenez-Alejandre, M., Alonso-Lopez, P., Farrera-Sal, M., Torres-Manjon, S., Ramos-Lozano, D., Moreno, R., Aerts, I., Doz, F., Cassoux, N., Chapeaublanc, E., Torrebadell, M., Roldan, M., Konig, A., Sunol, M., Claverol, J., Lavarino, C., Carmen de, T., Fu, L., Radvanyi, F., Munier, F.L., Catala-Mora, J., Mora, J., Alemany, R., Cascallo, M., Chantada, G.L., Carcaboso, A.M., 2019. Therapeutic targeting of the RB1 pathway in retinoblastoma with the oncolytic adenovirus VCN-01. *Sci Transl Med* 11.

Pascual-Pasto, G., Olaciregui, N.G., Vila-Ubach, M., Paco, S., Monterrubio, C., Rodriguez, E., Winter, U., Batalla-Vilacis, M., Catala, J., Salvador, H., Parareda, A., Schaiquevich, P., Sunol, M., Mora, J., Lavarino, C., de Torres, C., Chantada, G.L., Carcaboso, A.M., 2016. Preclinical platform of retinoblastoma xenografts recapitulating human disease and molecular markers of dissemination. *Cancer Lett* 380, 10-19.

Patel, S.R., Lin, A.S., Edelhauser, H.F., Prausnitz, M.R., 2011. Suprachoroidal drug delivery to the back of the eye using hollow microneedles. *Pharm Res* 28, 166-176.

Paulino, A.C., 1999. Trilateral retinoblastoma: is the location of the intracranial tumor important? *Cancer* 86, 135-141.

Pavlidou, E., Burris, C., Thaung, C., Scheimberg, I., Kingston, J., Hungerford, J.L., Reddy, M.A., Sagoo, M.S., 2015. Anterior Segment Seeding in Eyes With Retinoblastoma Failing to Respond to Intraophthalmic Artery Chemotherapy. *JAMA Ophthalmol* 133, 1455-1458.

Pawius, P., 1657. *Observatio XXIII. Tumor oculorum. Observations Anatomicae Selectiores*. Appendedto: Bartholinus T. *Historiarum Anatomicarum Rariorum, Centuria III & IV*. Copenhagen, Denmark. Petrus Morsing, 38-39.

Pham, T.T., Siebert, E., Asbach, P., Willerding, G., Erb-Eigner, K., 2015. Magnetic resonance imaging based morphologic evaluation of the pineal gland for suspected pineoblastoma in retinoblastoma patients and age-matched controls. *J Neurol Sci* 359, 185-192.

Phillips, T.J., McGuirk, S.P., Chahal, H.K., Kingston, J., Robertson, F., Brew, S., Roebuck, D., Hungerford, J.L., Herod, J., 2013. Autonomic cardio-respiratory reflex reactions and superselective ophthalmic arterial chemotherapy for retinoblastoma. *Paediatr Anaesth* 23, 940-945.

Pica, A., Moeckli, R., Balmer, A., Beck-Popovic, M., Chollet-Rivier, M., Do, H.P., Weber, D.C., Munier, F.L., 2011. Preliminary experience in treatment of papillary and macular retinoblastoma: evaluation of local control and local complications after treatment with linear accelerator-based stereotactic radiotherapy with micromultileaf collimator as second-line or salvage treatment after chemotherapy. *Int J Radiat Oncol Biol Phys* 81, 1380-1386.

Pontes de Carvalho, R.A., Krausse, M.L., Murphree, A.L., Schmitt, E.E., Campochiaro, P.A., Maumenee, I.H., 2006. Delivery from episcleral explants. *Invest Ophthalmol Vis Sci* 47, 4532-4539.

Popovic, M.B., Diezi, M., Kuchler, H., Abouzeid, H., Maeder, P., Balmer, A., Munier, F.L., 2007. Trilateral retinoblastoma with suprasellar tumor and associated pineal cyst. *J Pediatr Hematol Oncol* 29, 53-56.

Pratt, C.B., Crom, D.B., Howarth, C., 1985. The use of chemotherapy for extraocular retinoblastoma. *Med Pediatr Oncol* 13, 330-333.

Pratt, C.B., Meyer, D., Chenaille, P., Crom, D.B., 1989. The use of bone marrow aspirations and lumbar punctures at the time of diagnosis of retinoblastoma. *J Clin Oncol* 7, 140-143.

Pritchard, E.M., Dyer, M.A., Guy, R.K., 2016. Progress in Small Molecule Therapeutics for the Treatment of Retinoblastoma. *Mini Rev Med Chem* 16, 430-454.

Qaddoumi, I., Bass, J.K., Wu, J., Billups, C.A., Wozniak, A.W., Merchant, T.E., Haik, B.G., Wilson, M.W., Rodriguez-Galindo, C., 2012. Carboplatin-associated ototoxicity in children with retinoblastoma. *J Clin Oncol* 30, 1034-1041.

Racher, H., Soliman, S., Argiropoulos, B., Chan, H.S., Gallie, B.L., Perrier, R., Matevski, D., Rushlow, D., Piovesan, B., Shaikh, F., MacDonald, H., Corson, T.W., 2016. Molecular analysis distinguishes metastatic disease from second cancers in patients with retinoblastoma. *Cancer Genet* 209, 359-363.

Ramasubramanian, A., Kytasty, C., Meadows, A.T., Shields, J.A., Leahey, A., Shields, C.L., 2013. Incidence of pineal gland cyst and pineoblastoma in children with retinoblastoma during the chemoreduction era. *Am J Ophthalmol* 156, 825-829.

Rangamani, S., SathishKumar, K., Manoharan, N., Julka, P.K., Rath, G.K., Shanta, V., Swaminathan, R., Rama, R., Datta, K., Mandal, S., Koyande, S., Deshmane, V., Ganesh, B., Banavali, S.D., Badwe, R.A., Ramesh, C., Appaji, L., Nandakumar, A., 2015. Paediatric retinoblastoma in India: evidence from the National Cancer Registry Programme. *Asian Pac J Cancer Prev* 16, 4193-4198.

Rao, R., Honavar, S.G., Mulay, K., Reddy, V.A.P., 2018a. Eye salvage in diffuse anterior retinoblastoma using systemic chemotherapy with periocular and intravitreal topotecan. *J AAPOS* 22, 235-237 e232.

Rao, R., Honavar, S.G., Sharma, V., Reddy, V.A.P., 2018b. Intravitreal topotecan in the management of refractory and recurrent vitreous seeds in retinoblastoma. *Br J Ophthalmol* 102, 490-495.

Reddy, M.A., Naeem, Z., Duncan, C., Robertson, F., Herod, J., Rennie, A., Liasis, A., Thompson, D.A., Sagoo, M., 2017. Reduction of severe visual loss and complications following intra-arterial chemotherapy (IAC) for refractory retinoblastoma. *Br J Ophthalmol* 101, 1704-1708.

Reese, A.B., Ellsworth, R.M., 1963. The evaluation and current concept of retinoblastoma therapy. *Trans Am Acad Ophthalmol Otolaryngol* 67, 164-172.

Regan, D.P., Dubielzig, R.R., Zeiss, C.J., Charles, B., Hoy, S.S., Ehrhart, E.J., 2013. Primary primitive neuroectodermal tumors of the retina and ciliary body in dogs. *Vet Ophthalmol* 16 Suppl 1, 87-93.

Reid, T.W., Albert, D.M., Rabson, A.S., Russell, P., Craft, J., Chu, E.W., Tralka, T.S., Wilcox, J.L., 1974. Characteristics of an established cell line of retinoblastoma. *J Natl Cancer Inst* 53, 347-360.

Rimpela, A.K., Reinisalo, M., Hellinen, L., Grazhdankin, E., Kidron, H., Urtti, A., Del Amo, E.M., 2017. Implications of melanin binding in ocular drug delivery. *Adv Drug Deliv Rev*.

Robanus-Maandag, E., Dekker, M., van der Valk, M., Carrozza, M.L., Jeanny, J.C., Dannenberg, J.H., Berns, A., te Riele, H., 1998. p107 is a suppressor of retinoblastoma development in pRb-deficient mice. *Genes Dev* 12, 1599-1609.

Rodjan, F., de Graaf, P., Brisse, H.J., Goricke, S., Maeder, P., Galluzzi, P., Aerts, I., Alapetite, C., Desjardins, L., Wieland, R., Popovic, M.B., Diezi, M., Munier, F.L., Hadjistilianou, T., Knol, D.L., Moll, A.C., Castelijns, J.A., 2012. Trilateral retinoblastoma: neuroimaging characteristics and value of routine brain screening on admission. *J Neurooncol* 109, 535-544.

Rodjan, F., de Graaf, P., van der Valk, P., Hadjistilianou, T., Cerase, A., Toti, P., de Jong, M.C., Moll, A.C., Castelijns, J.A., Galluzzi, P., European Retinoblastoma Imaging, C., 2015. Detection of calcifications in retinoblastoma using gradient-echo MR imaging sequences: comparative study between in vivo MR imaging and ex vivo high-resolution CT. *AJNR Am J Neuroradiol* 36, 355-360.

Rodrigues, M.M., Rajagopalan, S., Lee, L., Nair, C.N., Advani, S.H., Donoso, L., Chader, G.J., Wiggert, B., 1992. Retinoblastoma: messenger RNA for interphotoreceptor retinoid binding protein. *Curr Eye Res* 11, 425-433.

Rodriguez-Galindo, C., Wilson, M.W., Haik, B.G., Merchant, T.E., Billups, C.A., Shah, N., Cain, A., Langston, J., Lipson, M., Kun, L.E., Pratt, C.B., 2003. Treatment of intraocular retinoblastoma with vincristine and carboplatin. *J Clin Oncol* 21, 2019-2025.

Rodriguez-Garcia, A., Gimenez-Alejandre, M., Rojas, J.J., Moreno, R., Bazan-Peregrino, M., Cascallo, M., Alemany, R., 2015. Safety and efficacy of VCN-01, an oncolytic adenovirus combining fiber HSG-binding domain replacement with RGD and hyaluronidase expression. *Clin Cancer Res* 21, 1406-1418.

Rojanaporn, D., Kaliki, S., Bianciotto, C.G., Iturralde, J.C., Say, E.A., Shields, C.L., 2012. Intravenous chemoreduction or intra-arterial chemotherapy for cavitary retinoblastoma: long-term results. *Arch Ophthalmol* 130, 585-590.

Rootman, D.B., Gonzalez, E., Mallipatna, A., Vandenhoven, C., Hampton, L., Dimaras, H., Chan, H.S., Gallie, B.L., Heon, E., 2013. Hand-held high-resolution spectral domain optical coherence tomography in retinoblastoma: clinical and morphologic considerations. *Br J Ophthalmol* 97, 59-65.

Ross, G., Lipper, E.G., Abramson, D., Preiser, L., 2001. The development of young children with retinoblastoma. *Arch Pediatr Adolesc Med* 155, 80-83.

Rowlands, M.A., Mondesire-Crump, I., Levin, A., Mauguen, A., Francis, J.H., Dunkel, I.J., Brodie, S.E., Gobin, Y.P., Abramson, D.H., 2018. Total retinal detachments due to retinoblastoma: Outcomes following intra-arterial chemotherapy/ophthalmic artery chemosurgery. *PLoS One* 13, e0195395.

Ruiz Del Rio, N., Abelairas Gomez, J.M., Alonso Garcia de la Rosa, F.J., Peralta Calvo, J.M., de Las Heras Martin, A., 2014. [Trilateral retinoblastoma. Correlation between the genetic anomalies of the RB1 gene and the presence of pineal gland cysts]. *Arch Soc Esp Oftalmol* 89, 4-9.

Rushlow, D.E., Mol, B.M., Kennett, J.Y., Yee, S., Pajovic, S., Theriault, B.L., Prigoda-Lee, N.L., Spencer, C., Dimaras, H., Corson, T.W., Pang, R., Massey, C., Godbout, R., Jiang, Z., Zacksenhaus, E., Paton, K., Moll, A.C., Houdayer, C., Raizis, A., Halliday, W., Lam, W.L., Boutros, P.C., Lohmann, D., Dorsman, J.C., Gallie, B.L., 2013. Characterisation of retinoblastomas without RB1 mutations: genomic, gene expression, and clinical studies. *Lancet Oncol* 14, 327-334.

Sahgal, A., Millar, B.A., Michaels, H., Jaywant, S., Chan, H.S., Heon, E., Gallie, B., Laperriere, N., 2006. Focal stereotactic external beam radiotherapy as a vision-sparing method for the treatment of peripapillary and perimacular retinoblastoma: preliminary results. *Clin Oncol (R Coll Radiol)* 18, 628-634.

Saktanasate, J., Vongkulsiri, S., Khoo, C.T., 2015. Invisible Retinoblastoma. *JAMA Ophthalmol* 133, e151123.

Saleh, R.A., Gross, S., Cassano, W., Gee, A., 1988. Metastatic retinoblastoma successfully treated with immunomagnetic purged autologous bone marrow transplantation. *Cancer* 62, 2301-2303.

Salim, A., Wiknjosastro, G.H., Danukusumo, D., Barnas, B., Zalud, I., 1998. Fetal retinoblastoma. *J Ultrasound Med* 17, 717-720.

Sampieri, K., Amenduni, M., Papa, F.T., Katzaki, E., Mencarelli, M.A., Marozza, A., Epistolato, M.C., Toti, P., Lazzi, S., Bruttini, M., De Filippis, R., De Francesco, S., Longo, I., Meloni, I., Mari, F., Acquaviva, A., Hadjistilianou, T., Renieri, A., Ariani, F., 2009. Array comparative genomic hybridization in retinoma and retinoblastoma tissues. *Cancer Sci* 100, 465-471.

Saremi, L., Imani, S., Rostaminia, M., Nadeali, Z., 2014. Parental age-related risk of retinoblastoma in Iranian children. *Asian Pac J Cancer Prev* 15, 2847-2850.

Sastre, X., Chantada, G.L., Doz, F., Wilson, M.W., de Davila, M.T., Rodriguez-Galindo, C., Chintagumpala, M., Chevez-Barrios, P., International Retinoblastoma Staging Working, G., 2009. Proceedings of the consensus meetings from the International Retinoblastoma Staging Working Group on the pathology guidelines for the examination of enucleated eyes and evaluation of prognostic risk factors in retinoblastoma. *Arch Pathol Lab Med* 133, 1199-1202.

Scelfo, C., Francis, J.H., Khetan, V., Jenkins, T., Marr, B., Abramson, D.H., Shields, C.L., Pe'er, J., Munier, F., Berry, J., Harbour, J.W., Yarovoy, A., Lucena, E., Murray, T.G., Bhagia, P., Paysse, E., Tuncer, S., Chantada, G.L., Moll, A.C., Ushakova, T., Plager, D.A., Ziyovuddin, I., Leal, C.A., Materin, M.A., Ji, X.D., Cursino, J.W., Polania, R., Kiratli, H., All-Ericsson, C., Kebudi, R., Honavar, S.G., Vishnevskia-Dai, V., Epelman, S., Daniels, A.B., Ling, J.D., Traore, F., Ramirez-Ortiz, M.A., 2017. An international survey of classification and treatment choices for group D retinoblastoma. *Int J Ophthalmol* 10, 961-967.

Schaiquevich, P., Buitrago, E., Ceciliano, A., Fandino, A.C., Asprea, M., Sierre, S., Abramson, D.H., Bramuglia, G.F., Chantada, G.L., 2012a. Pharmacokinetic analysis of topotecan after superselective ophthalmic artery infusion and periocular administration in a porcine model. *Retina* 32, 387-395.

Schaiquevich, P., Buitrago, E., Taich, P., Torbidoni, A., Ceciliano, A., Fandino, A., Asprea, M., Requejo, F., Abramson, D.H., Bramuglia, G.F., 2012b. Pharmacokinetic analysis of melphalan after superselective ophthalmic artery infusion in preclinical models and retinoblastoma patients. *Investigative ophthalmology & visual science* 53, 4205-4212.

Schaiquevich, P., Buitrago, E., Taich, P., Torbidoni, A., Ceciliano, A., Fandino, A., Asprea, M., Requejo, F., Abramson, D.H., Bramuglia, G.F., Chantada, G.L., 2012c. Pharmacokinetic analysis of melphalan after superselective ophthalmic artery infusion in preclinical models and retinoblastoma patients. *Invest Ophthalmol Vis Sci* 53, 4205-4212.

Schaiquevich, P., Ceciliano, A., Millan, N., Taich, P., Villasante, F., Fandino, A.C., Dominguez, J., Chantada, G.L., 2013. Intra-arterial chemotherapy is more effective than sequential periocular and intravenous chemotherapy as salvage treatment for relapsed retinoblastoma. *Pediatr Blood Cancer* 60, 766-770.

Schaiquevich, P., Fabius, A.W., Francis, J.H., Chantada, G.L., Abramson, D.H., 2017. Ocular Pharmacology of Chemotherapy for Retinoblastoma. *Retina* 37, 1-10.

Schedler, K.J., Traine, P.G., Lohmann, D.R., Haritoglou, C., Metz, K.A., Rodrigues, E.B., 2016. Hereditary Diffuse Infiltrating Retinoblastoma. *Ophthalmic Genet* 37, 95-97.

Scheffler, A.C., Cicciarelli, N., Feuer, W., Toledano, S., Murray, T.G., 2007. Macular retinoblastoma: evaluation of tumor control, local complications, and visual outcomes for eyes treated with chemotherapy and repetitive foveal laser ablation. *Ophthalmology* 114, 162-169.

Schiavetti, A., Hadjistilianou, T., Clerico, A., Bonci, E., Ragni, G., Castello, M.A., 2005. Conservative therapy in intraocular retinoblastoma: response/recurrence rate. *J Pediatr Hematol Oncol* 27, 3-6.

Schmack, I., Hubbard, G.B., Kang, S.J., Aaberg, T.M., Jr., Grossniklaus, H.E., 2006. Ischemic necrosis and atrophy of the optic nerve after periocular carboplatin injection for intraocular retinoblastoma. *Am J Ophthalmol* 142, 310-315.

Schueler, A.O., Fluhs, D., Anastassiou, G., Jurklies, C., Neuhauser, M., Schilling, H., Bornfeld, N., Sauerwein, W., 2006. Beta-ray brachytherapy with ¹⁰⁶Ru plaques for retinoblastoma. *Int J Radiat Oncol Biol Phys* 65, 1212-1221.

Schuler, A., Weber, S., Neuhauser, M., Jurklies, C., Lehnert, T., Heimann, H., Rudolph, G., Jockel, K.H., Bornfeld, N., Lohmann, D.R., 2005. Age at diagnosis of isolated unilateral retinoblastoma does not distinguish patients with and without a constitutional RB1 gene mutation but is influenced by a parent-of-origin effect. *Eur J Cancer* 41, 735-740.

- Scott, I.U., Murray, T.G., Feuer, W.J., Van Quill, K., Markoe, A.M., Ling, S., Roth, D.B., O'Brien, J.M., 1999. External beam radiotherapy in retinoblastoma: tumor control and comparison of 2 techniques. *Arch Ophthalmol* 117, 766-770.
- Seider, M.I., Grewal, D.S., Mruthyunjaya, P., 2016. Portable Optical Coherence Tomography Detection or Confirmation of Ophthalmoscopically Invisible or Indeterminate Active Retinoblastoma. *Ophthalmic Surg Lasers Imaging Retina* 47, 965-968.
- Seigel, G.M., Hackam, A.S., Ganguly, A., Mandell, L.M., Gonzalez-Fernandez, F., 2007. Human embryonic and neuronal stem cell markers in retinoblastoma. *Mol Vis* 13, 823-832.
- Semb, C., Erikson, H., Refsum, H.E., 1961. Ten years experience with tank respirators in the treatment of post-operative respiratory failure. *Acta Chir Scand Suppl* 283, 39-44.
- Semo, M., Vugler, A.A., Jeffery, G., 2007. Paradoxical opsin expressing cells in the inner retina that are augmented following retinal degeneration. *Eur J Neurosci* 25, 2296-2306.
- Sengupta, S., Pan, U., Khetan, V., 2016. Adult onset retinoblastoma. *Indian J Ophthalmol* 64, 485-491.
- Seregard, S., Kock, E., af Trampe, E., 1995. Intravitreal chemotherapy for recurrent retinoblastoma in an only eye. *Br J Ophthalmol* 79, 194-195.
- Seregard, S., Lundell, G., Svedberg, H., Kivelä, T., 2004. Incidence of retinoblastoma from 1958 to 1998 in Northern Europe: advantages of birth cohort analysis. *Ophthalmology* 111, 1228-1232.
- Sethi, R.V., Shih, H.A., Yeap, B.Y., Mouw, K.W., Petersen, R., Kim, D.Y., Munzenrider, J.E., Grabowski, E., Rodriguez-Galindo, C., Yock, T.I., Tarbell, N.J., Marcus, K.J., Mukai, S., MacDonald, S.M., 2014. Second nonocular tumors among survivors of retinoblastoma treated with contemporary photon and proton radiotherapy. *Cancer* 120, 126-133.
- Shah, P.K., Narendran, V., Kalpana, N., 2010. In vivo growth of retinoblastoma in a newborn infant. *Indian J Ophthalmol* 58, 421-423.
- Shah, P.K., Narendran, V., Manayath, G.J., Chowdhary, S., 2011. Atypical retinocytoma with diffuse vitreous seeds: An insight. *Oman J Ophthalmol* 4, 81-83.
- Sheen, V., Tucker, M.A., Abramson, D.H., Seddon, J.M., Kleinerman, R.A., 2008. Cancer screening practices of adult survivors of retinoblastoma at risk of second cancers. *Cancer* 113, 434-441.

Shen, H., Tang, Y., Xu, X., Tang, H., 2013. Detection of the GD2+/CD56+/CD45-immunophenotype by flow cytometry in cerebrospinal fluids from a patient with retinoblastoma. *Pediatr Hematol Oncol* 30, 30-32.

Sheppard, L., Eiser, C., Kingston, J., 2005. Mothers' perceptions of children's quality of life following early diagnosis and treatment for retinoblastoma (Rb). *Child Care Health Dev* 31, 137-142.

Sherr, C.J., McCormick, F., 2002. The RB and p53 pathways in cancer. *Cancer Cell* 2, 103-112.

Shields, C.L., Alset, A.E., Say, E.A., Caywood, E., Jabbour, P., Shields, J.A., 2016a. Retinoblastoma Control With Primary Intra-arterial Chemotherapy: Outcomes Before and During the Intravitreal Chemotherapy Era. *J Pediatr Ophthalmol Strabismus* 53, 275-284.

Shields, C.L., Bianciotto, C.G., Jabbour, P., Ramasubramanian, A., Lally, S.E., Griffin, G.C., Rosenwasser, R., Shields, J.A., 2011. Intra-arterial chemotherapy for retinoblastoma: report No. 1, control of retinal tumors, subretinal seeds, and vitreous seeds. *Arch Ophthalmol* 129, 1399-1406.

Shields, C.L., De Potter, P., Himelstein, B.P., Shields, J.A., Meadows, A.T., Maris, J.M., 1996. Chemoreduction in the initial management of intraocular retinoblastoma. *Arch Ophthalmol* 114, 1330-1338.

Shields, C.L., Douglass, A.M., Beggache, M., Say, E.A., Shields, J.A., 2016b. INTRAVITREOUS CHEMOTHERAPY FOR ACTIVE VITREOUS SEEDING FROM RETINOBLASTOMA: Outcomes After 192 Consecutive Injections. The 2015 Howard Naquin Lecture. *Retina* 36, 1184-1190.

Shields, C.L., Ghassemi, F., Tuncer, S., Thangappan, A., Shields, J.A., 2008. Clinical spectrum of diffuse infiltrating retinoblastoma in 34 consecutive eyes. *Ophthalmology* 115, 2253-2258.

Shields, C.L., Gorry, T., Shields, J.A., 2004a. Outcome of eyes with unilateral sporadic retinoblastoma based on the initial external findings by the family and the pediatrician. *J Pediatr Ophthalmol Strabismus* 41, 143-149; quiz 172-143.

Shields, C.L., Honavar, S., Shields, J.A., Demirci, H., Meadows, A.T., 2000. Vitrectomy in eyes with unsuspected retinoblastoma. *Ophthalmology* 107, 2250-2255.

Shields, C.L., Honavar, S.G., Meadows, A.T., Shields, J.A., Demirci, H., Singh, A., Friedman, D.L., Naduvilath, T.J., 2002. Chemoreduction plus focal therapy for retinoblastoma: factors predictive of need for treatment with external beam radiotherapy or enucleation. *Am J Ophthalmol* 133, 657-664.

Shields, C.L., Kaliki, S., Shah, S.U., Bianciotto, C.G., Jabbour, P., Shields, J.A., 2012. Effect of intraarterial chemotherapy on retinoblastoma-induced retinal detachment. *Retina* 32, 799-804.

Shields, C.L., Lally, S.E., Manjandavida, F.P., Leahey, A.M., Shields, J.A., 2016c. Diffuse Anterior Retinoblastoma with Globe Salvage and Visual Preservation in 3 Consecutive Cases. *Ophthalmology* 123, 378-384.

Shields, C.L., Manjandavida, F.P., Lally, S.E., Pieretti, G., Arepalli, S.A., Caywood, E.H., Jabbour, P., Shields, J.A., 2014. Intra-arterial chemotherapy for retinoblastoma in 70 eyes: outcomes based on the international classification of retinoblastoma. *Ophthalmology* 121, 1453-1460.

Shields, C.L., Mashayekhi, A., Au, A.K., Czyz, C., Leahey, A., Meadows, A.T., Shields, J.A., 2006a. The International Classification of Retinoblastoma predicts chemoreduction success. *Ophthalmology* 113, 2276-2280.

Shields, C.L., Mashayekhi, A., Cater, J., Shelil, A., Meadows, A.T., Shields, J.A., 2004b. Chemoreduction for retinoblastoma. Analysis of tumor control and risks for recurrence in 457 tumors. *Am J Ophthalmol* 138, 329-337.

Shields, C.L., Mashayekhi, A., Sun, H., Uysal, Y., Friere, J., Komarnicky, L., Shields, J.A., 2006b. Iodine 125 plaque radiotherapy as salvage treatment for retinoblastoma recurrence after chemoreduction in 84 tumors. *Ophthalmology* 113, 2087-2092.

Shields, C.L., Meadows, A.T., Shields, J.A., Carvalho, C., Smith, A.F., 2001. Chemoreduction for retinoblastoma may prevent intracranial neuroblastic malignancy (trilateral retinoblastoma). *Arch Ophthalmol* 119, 1269-1272.

Shields, C.L., Ramasubramanian, A., Thangappan, A., Hartzell, K., Leahey, A., Meadows, A.T., Shields, J.A., 2009. Chemoreduction for group E retinoblastoma: comparison of chemoreduction alone versus chemoreduction plus low-dose external radiotherapy in 76 eyes. *Ophthalmology* 116, 544-551 e541.

Shields, C.L., Santos, M.C., Diniz, W., Gunduz, K., Mercado, G., Cater, J.R., Shields, J.A., 1999. Thermotherapy for retinoblastoma. *Arch Ophthalmol* 117, 885-893.

Shields, C.L., Say, E.A., Pointdujour-Lim, R., Cao, C., Jabbour, P.M., Shields, J.A., 2015. Rescue intra-arterial chemotherapy following retinoblastoma recurrence after initial intra-arterial chemotherapy. *J Fr Ophtalmol* 38, 542-549.

Shields, C.L., Say, E.A.T., Pefkianaki, M., Regillo, C.D., Caywood, E.H., Jabbour, P.M., Shields, J.A., 2017. RHEGMATOGENOUS RETINAL DETACHMENT AFTER

INTRAAARTERIAL CHEMOTHERAPY FOR RETINOBLASTOMA: The 2016 Founders Award Lecture. *Retina* 37, 1441-1450.

Shields, C.L., Schoenberg, E., Kocher, K., Shukla, S.Y., Kaliki, S., Shields, J.A., 2013. Lesions simulating retinoblastoma (pseudoretinoblastoma) in 604 cases: results based on age at presentation. *Ophthalmology* 120, 311-316.

Shields, C.L., Shields, J.A., Baez, K.A., Cater, J., De Potter, P.V., 1993a. Choroidal invasion of retinoblastoma: metastatic potential and clinical risk factors. *Br J Ophthalmol* 77, 544-548.

Shields, C.L., Shields, J.A., Needle, M., de Potter, P., Kheterpal, S., Hamada, A., Meadows, A.T., 1997. Combined chemoreduction and adjuvant treatment for intraocular retinoblastoma. *Ophthalmology* 104, 2101-2111.

Shields, J.A., Demirci, H., Mashayekhi, A., Eagle, R.C., Jr., Shields, C.L., 2006c. Melanocytoma of the optic disk: a review. *Surv Ophthalmol* 51, 93-104.

Shields, J.A., Sanborn, G.E., Augsburger, J.J., Orlock, D., Donoso, L.A., 1982. Fluorescein angiography of retinoblastoma. *Trans Am Ophthalmol Soc* 80, 98-112.

Shields, J.A., Shields, C.L., De Potter, P., 1993b. Cryotherapy for retinoblastoma. *Int Ophthalmol Clin* 33, 101-105.

Shih, C.S., Laurie, N., Holzmacher, J., Spence, Y., Nathwani, A.C., Davidoff, A.M., Dyer, M.A., 2009. AAV-mediated local delivery of interferon-beta for the treatment of retinoblastoma in preclinical models. *Neuromolecular Med* 11, 43-52.

Shimoda, Y., Hamano, R., Ishihara, K., Shimoda, N., Hagimura, N., Akiyama, H., Kishi, S., Kaneko, A., 2008. Effects of intraocular irrigation with melphalan on rabbit retinas during vitrectomy. *Graefes Arch Clin Exp Ophthalmol* 246, 501-508.

Shin, J.Y., Kim, J.H., Yu, Y.S., Khwarg, S.I., Choung, H.K., Shin, H.Y., Ahn, H.S., 2010. Eye-preserving therapy in retinoblastoma: prolonged primary chemotherapy alone or combined with local therapy. *Korean J Ophthalmol* 24, 219-224.

Simpson, A.E., Gilbert, J.A., Rudnick, D.E., Geroski, D.H., Aaberg, T.M., Jr., Edelhauser, H.F., 2002. Transscleral diffusion of carboplatin: an in vitro and in vivo study. *Arch Ophthalmol* 120, 1069-1074.

Singh, A.D., Santos, C.M., Shields, C.L., Shields, J.A., Eagle, R.C., Jr., 2000. Observations on 17 patients with retinocytoma. *Arch Ophthalmol* 118, 199-205.

- Singh, H., Koos, D., Fernandez, E., Thornton, M.E., Grubbs, B.H., Moats, R., Fraser, S., Cobrinik, D., 2018a. Precursor proliferation and inward migration in intact cultured retina. *Investigative Ophthalmology & Visual Science (IOVS)* 59, 4961-4961.
- Singh, H.P., Wang, S., Cobrinik, D., 2016. Human cone precursor program underlying a proliferative response to pRB loss. *Investigative ophthalmology & visual science* 57, 556.
- Singh, H.P., Wang, S., Stachelek, K., Lee, S., Reid, M.W., Thornton, M.E., Craft, C.M., Grubbs, B.H., Cobrinik, D., 2018b. Developmental stage-specific proliferation and retinoblastoma genesis in RB-deficient human but not mouse cone precursors. *Proc Natl Acad Sci U S A* 115, E9391-E9400.
- Singh PH, Thornton M, Grubbs B, Cobrinik D. MYCN overexpression induces cone precursor proliferation and tumorigenesis in explanted retinæ. *Invest Ophthalmol Vis Sci*. 2019;60:ARVO E-Abstract 1320
- Sippel, K.C., Fraioli, R.E., Smith, G.D., Schalkoff, M.E., Sutherland, J., Gallie, B.L., Dryja, T.P., 1998. Frequency of somatic and germ-line mosaicism in retinoblastoma: implications for genetic counseling. *Am J Hum Genet* 62, 610-619.
- Sirin, S., de Jong, M.C., Galluzzi, P., Maeder, P., Brisse, H.J., Castelijns, J.A., de Graaf, P., Goerickx, S.L., European Retinoblastoma Imaging, C., 2016. MRI-based assessment of the pineal gland in a large population of children aged 0-5 years and comparison with pineoblastoma: part II, the cystic gland. *Neuroradiology* 58, 713-721.
- Smith, S.J., Smith, B.D., 2013. Evaluating the risk of extraocular tumour spread following intravitreal injection therapy for retinoblastoma: a systematic review. *Br J Ophthalmol* 97, 1231-1236.
- Smith, S.J., Smith, B.D., Mohny, B.G., 2014. Ocular side effects following intravitreal injection therapy for retinoblastoma: a systematic review. *Br J Ophthalmol* 98, 292-297.
- Soliman, S.E., D'Silva, C.N., Dimaras, H., Dzneldze, I., Chan, H., Gallie, B.L., 2018. Clinical and genetic associations for carboplatin-related ototoxicity in children treated for retinoblastoma: A retrospective noncomparative single-institute experience. *Pediatr Blood Cancer* 65, e26931.
- Soliman, S.E., Dimaras, H., Khetan, V., Gardiner, J.A., Chan, H.S., Heon, E., Gallie, B.L., 2016. Prenatal versus Postnatal Screening for Familial Retinoblastoma. *Ophthalmology* 123, 2610-2617.

Soliman, S.E., VandenHoven, C., MacKeen, L.D., Heon, E., Gallie, B.L., 2017. Optical Coherence Tomography-Guided Decisions in Retinoblastoma Management. *Ophthalmology* 124, 859-872.

Song, X., Zhou, Y., Jia, R., Xu, X., Wang, H., Hu, J., Ge, S., Fan, X., 2010. Inhibition of retinoblastoma in vitro and in vivo with conditionally replicating oncolytic adenovirus H101. *Invest Ophthalmol Vis Sci* 51, 2626-2635.

Spaulding, A.G., Fuhs, J.C., 1968. Retinoblastoma and retinal detachment. *Surv Ophthalmol* 13, 152-156.

Spencer, C., Pajovic, S., Devlin, H., Dinh, Q.D., Corson, T.W., Gallie, B.L., 2005. Distinct patterns of expression of the RB gene family in mouse and human retina. *Gene Expr Patterns* 5, 687-694.

Sreelakshmi, K.V., Chandra, A., Krishnakumar, S., Natarajan, V., Khetan, V., 2017. Anterior Chamber Invasion in Retinoblastoma: Not an Indication for Adjuvant Chemotherapy. *Invest Ophthalmol Vis Sci* 58, 4654-4661.

Stacey, A.W., Clarke, B., Moraitis, C., Fabian, I.D., Smith, V., Sagoo, M.S., Reddy, M.A., 2019. The Incidence of Binocular Visual Impairment and Blindness in Children with Bilateral Retinoblastoma. *Ocul Oncol Pathol* 5, 1-7.

Staffieri, S.E., McGillivray, G., Elder, J.E., Bristowe, A., Cole, S., McKenzie, J.D., Fink, A.M., 2015. Managing fetuses at high risk of retinoblastoma: lesion detection on screening MRI. *Prenat Diagn* 35, 174-178.

Stathopoulos, C., Gaillard, M.C., Moulin, A., Puccinelli, F., Beck-Popovic, M., Munier, F.L., 2018a. INTRAVITREAL ANTI-VASCULAR ENDOTHELIAL GROWTH FACTOR FOR THE MANAGEMENT OF NEOVASCULARIZATION IN RETINOBLASTOMA AFTER INTRAVENOUS AND/OR INTRAARTERIAL CHEMOTHERAPY: Long-Term Outcomes in a Series of 35 Eyes. *Retina*.

Stathopoulos, C., Gaillard, M.C., Puccinelli, F., Maeder, P., Hadjistilianou, D., Beck-Popovic, M., Munier, F.L., 2018b. Successful conservative treatment of massive choroidal relapse in 2 retinoblastoma patients monitored by ultrasound biomicroscopy and/or spectral domain optic coherence tomography. *Ophthalmic Genet* 39, 242-246.

Stathopoulos, C., Moulin, A., Gaillard, M.C., Beck-Popovic, M., Puccinelli, F., Munier, F.L., 2018c. Conservative treatment of diffuse infiltrating retinoblastoma: optical coherence tomography-assisted diagnosis and follow-up in three consecutive cases. *Br J Ophthalmol*.

Stathopoulos, C., Say, E.A.T., Shields, C.L., 2018d. Prenatal ultrasonographic detection and prenatal (prior to birth) management of hereditary retinoblastoma. *Graefes Arch Clin Exp Ophthalmol* 256, 861-862.

Steinle, J.J., Zhang, Q., Thompson, K.E., Toutounchian, J., Yates, C.R., Soderland, C., Wang, F., Stewart, C.F., Haik, B.G., Williams, J.S., Jackson, J.S., Mandrell, T.D., Johnson, D., Wilson, M.W., 2012. Intra-ophthalmic artery chemotherapy triggers vascular toxicity through endothelial cell inflammation and leukostasis. *Invest Ophthalmol Vis Sci* 53, 2439-2445.

Steliarova-Foucher, E., Colombet, M., Ries, L.A.G., Moreno, F., Dolya, A., Bray, F., Hesselting, P., Shin, H.Y., Stiller, C.A., contributors, I.-. 2017. International incidence of childhood cancer, 2001-10: a population-based registry study. *Lancet Oncol* 18, 719-731.

Suryawanshi, P., Ramadwar, M., Dikshit, R., Kane, S.V., Kurkure, P., Banavali, S., Viswanathan, S., 2011. A study of pathologic risk factors in postchemoreduced, enucleated specimens of advanced retinoblastomas in a developing country. *Arch Pathol Lab Med* 135, 1017-1023.

Süsskind, D., Hagemann, U., Schrader, M., Januschowski, K., Schnichels, S., Aisenbrey, S., 2016. Toxic effects of melphalan, topotecan and carboplatin on retinal pigment epithelial cells. *Acta ophthalmologica* 94, 471-478.

Suzuki, S., Aihara, Y., Fujiwara, M., Sano, S., Kaneko, A., 2015. Intravitreal injection of melphalan for intraocular retinoblastoma. *Jpn J Ophthalmol* 59, 164-172.

Suzuki, S., Yamane, T., Mohri, M., Kaneko, A., 2011. Selective ophthalmic arterial injection therapy for intraocular retinoblastoma: the long-term prognosis. *Ophthalmology* 118, 2081-2087.

Syed, N.A., Nork, T.M., Poulsen, G.L., Riis, R.C., George, C., Albert, D.M., 1997. Retinoblastoma in a dog. *Arch Ophthalmol* 115, 758-763.

Taich, P., Ceciliano, A., Buitrago, E., Sampor, C., Fandino, A., Villasante, F., Lucena, E., Romero, L., Chantada, G.L., Schaiquevich, P., 2014. Clinical pharmacokinetics of intra-arterial melphalan and topotecan combination in patients with retinoblastoma. *Ophthalmology* 121, 889-897.

Taich, P., Requejo, F., Asprea, M., Sgroi, M., Gobin, P., Abramson, D.H., Chantada, G., Schaiquevich, P., 2016. Topotecan Delivery to the Optic Nerve after Ophthalmic Artery Chemosurgery. *PLoS One* 11, e0151343.

Tawansy, K.A., Samuel, M.A., Shammass, M., Murphree, A.L., 2006. Vitreoretinal complications of retinoblastoma treatment. *Retina* 26, S47-52.

Teichmann, K.D., 2002. Intravitreal injections: does globe size matter? *J Cataract Refract Surg* 28, 1886-1889.

Temming, P., Arendt, M., Viehmann, A., Eisele, L., Le Guin, C.H., Schundeln, M.M., Biewald, E., Astrahantseff, K., Wieland, R., Bornfeld, N., Sauerwein, W., Eggert, A., Jockel, K.H., Lohmann, D.R., 2017. Incidence of second cancers after radiotherapy and systemic chemotherapy in heritable retinoblastoma survivors: A report from the German reference center. *Pediatr Blood Cancer* 64, 71-80.

Temming, P., Viehmann, A., Arendt, M., Eisele, L., Spix, C., Bornfeld, N., Sauerwein, W., Jockel, K.H., Lohmann, D.R., 2015. Pediatric second primary malignancies after retinoblastoma treatment. *Pediatr Blood Cancer* 62, 1799-1804.

Thampi, S., Hetts, S.W., Cooke, D.L., Stewart, P.J., Robbins, E., Banerjee, A., Dubois, S.G., Char, D., Halbach, V., Matthay, K., 2013. Superselective intra-arterial melphalan therapy for newly diagnosed and refractory retinoblastoma: results from a single institution. *Clin Ophthalmol* 7, 981-989.

Toi, A., Sutherland, J., Gallie, B., Gardiner, J., Sermer, M., 2003. Evaluation of the fetus at risk for retinoblastoma: what is the role of prenatal ultrasound? *Ultrasound in Medicine & Biology* 29, S137.

Torbidoni, A.V., Laurent, V.E., Sampor, C., Ottaviani, D., Vazquez, V., Gabri, M.R., Rossi, J., de Davila, M.T., Alonso, C., Alonso, D.F., Chantada, G.L., 2015a. Association of Cone-Rod Homeobox Transcription Factor Messenger RNA With Pediatric Metastatic Retinoblastoma. *JAMA Ophthalmol* 133, 805-812.

Torbidoni, A.V., Laurent, V.E., Sampor, C., Ottaviani, D., Vazquez, V., Gabri, M.R., Rossi, J., de Davila, M.T., Alonso, C., Alonso, D.F., Chantada, G.L., 2015b. Association of Cone-Rod Homeobox Transcription Factor Messenger RNA With Pediatric Metastatic Retinoblastoma. *JAMA Ophthalmol*.

Torbidoni, A.V., Sampor, C., Laurent, V.E., Aschero, R., Iyer, S., Rossi, J., Alderete, D., Alonso, D.F., Szijan, I., Chantada, G.L., 2018. Minimal disseminated disease evaluation and outcome in trilateral retinoblastoma. *Br J Ophthalmol* 102, 1597-1601.

Traine, P.G., Schedler, K.J., Rodrigues, E.B., 2016. Clinical Presentation and Genetic Paradigm of Diffuse Infiltrating Retinoblastoma: A Review. *Ocul Oncol Pathol* 2, 128-132.

Tran, H.V., Schorderet, D.F., Gaillard, M.C., Balmer, A., Munier, F.L., 2012. Risk assessment of recurrence in sporadic retinoblastoma using a molecular-based algorithm. *Ophthalmic Genet* 33, 6-11.

Tse, B.C., Steinle, J.J., Johnson, D., Haik, B.G., Wilson, M.W., 2013. Superselective intraophthalmic artery chemotherapy in a nonhuman primate model: histopathologic findings. *JAMA Ophthalmol* 131, 903-911.

Tsimpida, M., Thompson, D.A., Liasis, A., Smith, V., Kingston, J.E., Sagoo, M.S., Reddy, M.A., 2013. Visual outcomes following intraophthalmic artery melphalan for patients with refractory retinoblastoma and age appropriate vision. *Br J Ophthalmol* 97, 1464-1470.

Tuncer, S., Sencer, S., Kebudi, R., Tanyildiz, B., Cebeci, Z., Aydin, K., 2016. Superselective intra-arterial chemotherapy in the primary management of advanced intra-ocular retinoblastoma: first 4-year experience from a single institution in Turkey. *Acta Ophthalmol* 94, e644-e651.

Turaka, K., Shields, C.L., Meadows, A.T., Leahey, A., 2012. Second malignant neoplasms following chemoreduction with carboplatin, etoposide, and vincristine in 245 patients with intraocular retinoblastoma. *Pediatr Blood Cancer* 59, 121-125.

Ueda, M., Tanabe, J., Inomata, M., Kaneko, A., Kimura, T., 1995. [Study on conservative treatment of retinoblastoma--effect of intravitreal injection of melphalan on the rabbit retina]. *Nippon Ganka Gakkai Zasshi* 99, 1230-1235.

United Nations, Department of Economic and Social Affairs, Population Division, 2017. *World Population Prospects: The 2017 Revision*.

Usmanov, R.H., Kivela, T., 2014. Predicted Trends in the Incidence of Retinoblastoma in the Asia-Pacific Region. *Asia Pac J Ophthalmol (Phila)* 3, 151-157.

Uusitalo, M.S., Van Quill, K.R., Scott, I.U., Matthay, K.K., Murray, T.G., O'Brien, J.M., 2001. Evaluation of chemoprophylaxis in patients with unilateral retinoblastoma with high-risk features on histopathologic examination. *Arch Ophthalmol* 119, 41-48.

Valverde, J.R., Alonso, J., Palacios, I., Pestana, A., 2005. RB1 gene mutation up-date, a meta-analysis based on 932 reported mutations available in a searchable database. *BMC Genet* 6, 53.

van Dijk, J., Grootenhuys, M.A., Imhof, S.M., Cohen-Kettenis, P.T., Moll, A.C., Huisman, J., 2009. Coping strategies of retinoblastoma survivors in relation to behavioural problems. *Psychooncology* 18, 1281-1289.

van Dijk, J., Huisman, J., Moll, A.C., Schouten-van Meeteren, A.Y., Bezemer, P.D., Ringens, P.J., Cohen-Kettenis, P.T., Imhof, S.M., 2007a. Health-related quality of life of child and adolescent retinoblastoma survivors in the Netherlands. *Health Qual Life Outcomes* 5, 65.

van Dijk, J., Imhof, S.M., Moll, A.C., Ringens, P.J., Cohen-Kettenis, P.T., Rijmen, F., Huisman, J., 2007b. Quality of life of adult retinoblastoma survivors in the Netherlands. *Health Qual Life Outcomes* 5, 30.

van Dijk, J., Oostrom, K.J., Huisman, J., Moll, A.C., Cohen-Kettenis, P.T., Ringens, P.J., Imhof, S.M., 2010. Restrictions in daily life after retinoblastoma from the perspective of the survivors. *Pediatr Blood Cancer* 54, 110-115.

Vasilatou-Kosmidis, H., 2003. Cancer in neonates and infants. *Med Pediatr Oncol* 41, 7-9.

Vasquez, L.M., Giuliani, G.P., Halliday, W., Pavlin, C.J., Gallie, B.L., Heon, E., 2011. Ultrasound biomicroscopy in the management of retinoblastoma. *Eye (Lond)* 25, 141-147.

Venturi, C., Bracco, S., Cerase, A., Cioni, S., Galluzzi, P., Gennari, P., Vallone, I.M., Tinturini, R., Vittori, C., De Francesco, S., Caini, M., D'Ambrosio, A., Toti, P., Renieri, A., Hadjistilianou, T., 2013. Superselective ophthalmic artery infusion of melphalan for intraocular retinoblastoma: preliminary results from 140 treatments. *Acta Ophthalmol* 91, 335-342.

Verhoeff, F.H., Jackson, E., 1926. Minutes of the proceedings, 62nd annual meeting, pp. 38-43.

Vézina, M., 2013. Comparative Ocular Anatomy in Commonly Used Laboratory Animals, in: Weir, B.A., Collins, M. (Eds.), *Assessing Ocular Toxicology in Laboratory Animals*. Humana Press, Totowa, NJ, pp. 1-21.

Vogelstein, B., Fearon, E.R., Hamilton, S.R., Kern, S.E., Preisinger, A.C., Leppert, M., Nakamura, Y., White, R., Smits, A.M., Bos, J.L., 1988. Genetic alterations during colorectal-tumor development. *N Engl J Med* 319, 525-532.

Waddell, K.M., Kagame, K., Ndamira, A., Twinamasiko, A., Picton, S.V., Simmons, I.G., Johnston, W.T., Newton, R., 2015. Clinical features and survival among children with retinoblastoma in Uganda. *Br J Ophthalmol* 99, 387-390.

Wallach, M., Balmer, A., Munier, F., Houghton, S., Pampallona, S., von der Weid, N., Beck-Popovic, M., Swiss Pediatric Oncology, G., Swiss Childhood Cancer, R., 2006. Shorter time to diagnosis and improved stage at presentation in Swiss patients with retinoblastoma treated from 1963 to 2004. *Pediatrics* 118, e1493-1498.

- Wang, H., Wei, F., Li, H., Ji, X., Li, S., Chen, X., 2013. Combination of oncolytic adenovirus and endostatin inhibits human retinoblastoma in an in vivo mouse model. *Int J Mol Med* 31, 377-385.
- Warden, S.M., Mukai, S., 2006. Pars plana vitrectomy in eyes treated for retinoblastoma. *Retina* 26, S53-56.
- Watts, P., Westall, C., Colpa, L., MacKeen, L., Abdoell, M., Gallie, B., Heon, E., 2002. Visual results in children treated for macular retinoblastoma. *Eye (Lond)* 16, 75-80.
- Weintraub, N., Rot, I., Shoshani, N., Pe'er, J., Weintraub, M., 2011. Participation in daily activities and quality of life in survivors of retinoblastoma. *Pediatr Blood Cancer* 56, 590-594.
- Weiss, A.H., Karr, D.J., Kalina, R.E., Lindsley, K.L., Pendergrass, T.W., 1994. Visual outcomes of macular retinoblastoma after external beam radiation therapy. *Ophthalmology* 101, 1244-1249.
- Whitehead, M.T., Oh, C.C., Choudhri, A.F., 2013a. Incidental pineal cysts in children who undergo 3-T MRI. *Pediatr Radiol* 43, 1577-1583.
- Whitehead, M.T., Oh, C.C., Choudhri, A.F., 2013b. Lu-Incidental pineal cysts in children who undergo 3-T MRI. *Pediatr Radiol* 43, 1577-1583.
- Williams, R.W., 1991. The human retina has a cone-enriched rim. *Vis Neurosci* 6, 403-406.
- Wilson, M.W., Haik, B.G., Liu, T., Merchant, T.E., Rodriguez-Galindo, C., 2005. Effect on ocular survival of adding early intensive focal treatments to a two-drug chemotherapy regimen in patients with retinoblastoma. *Am J Ophthalmol* 140, 397-406.
- Wilson, M.W., Jackson, J.S., Phillips, B.X., Buchanan, J., Frase, S., Wang, F., Steinle, J.J., Stewart, C.F., Mandrell, T.D., Haik, B.G., Williams, J.S., 2011. Real-time ophthalmoscopic findings of superselective intraophthalmic artery chemotherapy in a nonhuman primate model. *Arch Ophthalmol* 129, 1458-1465.
- Wilson, M.W., Rodriguez-Galindo, C., Haik, B.G., Moshfeghi, D.M., Merchant, T.E., Pratt, C.B., 2001. Multiagent chemotherapy as neoadjuvant treatment for multifocal intraocular retinoblastoma. *Ophthalmology* 108, 2106-2114; discussion 2114-2105.
- Wilson, T.W., Chan, H.S., Moselhy, G.M., Heydt, D.D., Jr., Frey, C.M., Gallie, B.L., 1996. Penetration of chemotherapy into vitreous is increased by cryotherapy and cyclosporine in rabbits. *Arch Ophthalmol* 114, 1390-1395.

- Winter, U., Nicolas, M., Sgroi, M., Sampor, C., Torbidoni, A., Fandino, A., Chantada, G.L., Munier, F.L., Schaiquevich, P., 2018. Assessment of retinoblastoma RNA reflux after intravitreal injection of melphalan. *Br J Ophthalmol* 102, 415-418.
- Wintersteiner, H., 1897. Das neuroepithelioma Retinae; eine anatomische und klinische Studie. Deuticke.
- Wong, F.L., Boice, J.D., Jr., Abramson, D.H., Tarone, R.E., Kleinerman, R.A., Stovall, M., Goldman, M.B., Seddon, J.M., Tarbell, N., Fraumeni, J.F., Jr., Li, F.P., 1997. Cancer incidence after retinoblastoma. Radiation dose and sarcoma risk. *JAMA* 278, 1262-1267.
- Woo, K.I., Harbour, J.W., 2010. Review of 676 second primary tumors in patients with retinoblastoma: association between age at onset and tumor type. *Arch Ophthalmol* 128, 865-870.
- Wu, N., Jia, D., Bates, B., Basom, R., Eberhart, C.G., MacPherson, D., 2017. A mouse model of MYCN-driven retinoblastoma reveals MYCN-independent tumor reemergence. *J Clin Invest* 127, 888-898.
- Wyse, E., Handa, J.T., Friedman, A.D., Pearl, M.S., 2016. A review of the literature for intra-arterial chemotherapy used to treat retinoblastoma. *Pediatr Radiol* 46, 1223-1233.
- Xu, X.L., Fang, Y., Lee, T.C., Forrest, D., Gregory-Evans, C., Almeida, D., Liu, A., Jhanwar, S.C., Abramson, D.H., Cobrinik, D., 2009. Retinoblastoma has properties of a cone precursor tumor and depends upon cone-specific MDM2 signaling. *Cell* 137, 1018-1031.
- Xu, X.L., Lee, T.C., Offor, N., Cheng, C., Liu, A., Fang, Y., Jhanwar, S.C., Abramson, D.H., Cobrinik, D., 2010. Tumor-associated retinal astrocytes promote retinoblastoma cell proliferation through production of IGFBP-5. *Am J Pathol* 177, 424-435.
- Xu, X.L., Singh, H.P., Wang, L., Qi, D.L., Poulos, B.K., Abramson, D.H., Jhanwar, S.C., Cobrinik, D., 2014. Rb suppresses human cone-precursor-derived retinoblastoma tumours. *Nature* 514, 385-388.
- Yamane, T., Kaneko, A., Mohri, M., 2004. The technique of ophthalmic arterial infusion therapy for patients with intraocular retinoblastoma. *Int J Clin Oncol* 9, 69-73.
- Yannuzzi, N.A., Francis, J.H., Marr, B.P., Belinsky, I., Dunkel, I.J., Gobin, Y.P., Abramson, D.H., 2015. Enucleation vs Ophthalmic Artery Chemosurgery for Advanced Intraocular Retinoblastoma: A Retrospective Analysis. *JAMA Ophthalmol* 133, 1062-1066.

Yarovoy, A.A., Ushakova, T.L., Gorshkov, I.M., Polyakov, V.G., Golubeva, O.V., Gorovtsova, O.V., Krivoviyaz, O.S., 2015. Intraocular surgery with melphalan irrigation for vitreous hemorrhage in an only eye with retinoblastoma. *Eur J Ophthalmol* 26, e17-19.

Yip, B.H., Pawitan, Y., Czene, K., 2006. Parental age and risk of childhood cancers: a population-based cohort study from Sweden. *Int J Epidemiol* 35, 1495-1503.

Yousef, Y.A., Soliman, S.E., Astudillo, P.P., Durairaj, P., Dimaras, H., Chan, H.S., Heon, E., Gallie, B.L., Shaikh, F., 2016. Intra-arterial Chemotherapy for Retinoblastoma: A Systematic Review. *JAMA Ophthalmol*.

Yu, C.L., Tucker, M.A., Abramson, D.H., Furukawa, K., Seddon, J.M., Stovall, M., Fraumeni, J.F., Jr., Kleinerman, R.A., 2009. Cause-specific mortality in long-term survivors of retinoblastoma. *J Natl Cancer Inst* 101, 581-591.

Zhang, J., Benavente, C.A., McEvoy, J., Flores-Otero, J., Ding, L., Chen, X., Ulyanov, A., Wu, G., Wilson, M., Wang, J., Brennan, R., Rusch, M., Manning, A.L., Ma, J., Easton, J., Shurtleff, S., Mullighan, C., Pounds, S., Mukatira, S., Gupta, P., Neale, G., Zhao, D., Lu, C., Fulton, R.S., Fulton, L.L., Hong, X., Dooling, D.J., Ochoa, K., Naeve, C., Dyson, N.J., Mardis, E.R., Bahrami, A., Ellison, D., Wilson, R.K., Downing, J.R., Dyer, M.A., 2012. A novel retinoblastoma therapy from genomic and epigenetic analyses. *Nature* 481, 329-334.

Zhao, J., Dimaras, H., Massey, C., Xu, X., Huang, D., Li, B., Chan, H.S., Gallie, B.L., 2011. Pre-nucleation chemotherapy for eyes severely affected by retinoblastoma masks risk of tumor extension and increases death from metastasis. *J Clin Oncol* 29, 845-851.

Zhu, X., Dunn, J.M., Goddard, A.D., Squire, J.A., Becker, A., Phillips, R.A., Gallie, B.L., 1992. Mechanisms of loss of heterozygosity in retinoblastoma. *Cytogenet Cell Genet* 59, 248-252.

Zhu, D., Berry, J.L., Ediriwickrema, L., Wong, K., Lee, T.C., Murphree, A.L., Kim, J.W., Jubran, R., 2015. Long-Term Outcomes of Group B Eyes in Patients with Retinoblastoma Treated with Short-Course Chemoreduction: Experience from Children's Hospital Los Angeles/University of Southern California. *Ocul Oncol Pathol* 2, 105-111.

Zhu, X.P., Dunn, J.M., Phillips, R.A., Goddard, A.D., Paton, K.E., Becker, A., Gallie, B.L., 1989. Preferential germline mutation of the paternal allele in retinoblastoma. *Nature* 340, 312-313.

Web references

United Nations, Department of Economic and Social Affairs, Population Division, 2017. World Population Prospects: The 2017 Revision. <https://esa.un.org/unpd/wpp/>

Children's Oncology Group. Newly diagnosed with retinoblastoma. Children's Oncology Group [online], <http://www.childrensoncologygroup.org/index.php/newlydiagnosedwithretinoblastoma> (2011).

<http://grantome.com/grant/NIH/RC3-CA150730-01>

Legends to figures

Figure 1. Incidence and predicted incidence of retinoblastoma from 1960 to 2060. **A.** In the world and by region **(B)** Africa, **(C)** Asia, **(D)** Europe, **(E)** Latin America, and **(F)** North America. The population, birth rate and infant mortality are taken from the World Population Prospects, 2017 Revision (United Nations, 2017), and the number of retinoblastomas per live born children from a North European birth cohort estimates (Seregard et al., 2004). Vertical lines indicate 2015. Undulations and peaks in the graphs are primarily explained by changes in birth rates.

Figure 2. Gaze-dependent leukocoria as presenting sign of a retinoblastoma in the left eye of a 14-month-old boy not visible on straight gaze but only from the temporal side **(A)** due to the nasal location of the tumor **(B)**.

Figure 3. Fundus extremicus. A-B. Schematic representation of the normal microanatomy of the retinal extreme periphery. **A.** En face view (dash line = longitudinal section) and **(B)** longitudinal section **C.** Photograph of peripheral infero-nasal retinal indentation showing retinal oral teeth (13) and an isolated ciliary retinal island (12). **D.** Longitudinal UBM (ultrasonographic biomicroscopy) of the periphery (50 MHz).

1. Choroid 2. Retina 3. Internal limiting membrane 4. Posterior hyaloid. 5. Peripheral retina posterior to the vitreous base 6. Posterior limit of the vitreous base 7. Retinal vitreous base with fenestrated inner limiting membrane and devoid of overlying hyaloid 8. Ora serrata and posterior insertion of the anterior hyaloid 9. Cone-enriched rim 10. Anterior hyaloid 11. Pars plana. 12. Isolated ciliary retinal island (ectopic retina) devoid of inner limiting membrane 13. Retinal oral tooth devoid of inner limiting membrane 14. Pars plicata. 15. Posterior chamber 16. Anterior chamber 17. Hannover's canal 18. Petit's canal 19. Vitreous.

Figure 4. Role of optical coherence tomography (OCT) in the management of retinoblastoma. **A.** Detection of an invisible parapapillary tumor in the left eye of a 2-month-old girl referred for a familial retinoblastoma (scT1bN0M0), **(B)** which regressed to a hyperreflective scar on OCT after Argon laser treatment. **C.** Early diagnosis of a recurrence over a parapapillary scar. **D.** Diagnosis of an invisible epipapillary vitreous seed. Note the intact demarcation line between the seed and the papilla, supporting the absence of prelaminar invasion, before **(D)** and after **(E)** treatment with intravitreal melphalan. **F.** Documentation of an intact fovea behind dense epiretinal seeding predictive of a good visual prognosis. **G.** Assessment of the treatment response of prehyaloidal vitreous seeds (spheres) before **(G)** and after **(H)** intravitreal melphalan. The appearance of hyperreflective dots in spheres indicates complete response implying treatment completion. **I.** Early diagnosis of a secondary choroidal invasion. **J.** Posterior pole retinoblastoma (arrow heads) at diagnosis and **(K)** after 6 cycles of systemic chemotherapy in the context of bilateral disease, showing regression type 0 on indirect ophthalmoscopy, but persistent lesion on OCT necessitating appropriate follow-up. **L-N.** Two-week-old girl with leukocoria, neovascular glaucoma and total exudative retinal detachment in the right eye **(L)** referred for suspicion of retinoblastoma. **M, N.** Left eye examination revealed multiple small retinal lesions involving the nerve fiber layer on OCT, corresponding to retinal astrocytic hamartomas. Tuberous sclerosis was confirmed on magnetic resonance imaging one week later, and by the presence of a germline *TSC2* mutation. Pathology of the enucleated right eye revealed a giant cell astrocytoma.

NB: *Lines on fundus photography correspond to the adjacent optical coherence tomography scan.*

Figure 5. Magnetic Resonance Imaging of the pineal gland. **A.** Pineal trilateral retinoblastoma: T1 sagittal image after injection of gadolinium in a 19-month-old girl with bilateral retinoblastoma **B.** Suprasellar trilateral retinoblastoma: T1 sagittal image after injection of gadolinium in a 14-month-old boy, showing a cystic and nodular pineal gland and a large tumor in the pituitary sella. **C.** T1 sagittal image after injection of gadolinium showing a pineal nodule in the posterior wall of a cystic gland in a 45-month-old boy, growing to pinealoblastoma three months later (**D**). **E-F.** T2 sagittal TSE (Turbo spin echo) image in a 4 months old patient with bilateral retinoblastoma showing the growth of a pineal 2a cyst over 15 months, followed by a gradual regression over 4 years (**G**).

Figure 6. A. Prenatal detection of a macular tumor in the left eye by transabdominal ultrasonography (5 MHz) at 33 weeks of gestation. **B.** Postnatal ultrasonography (10 MHz) obtained after provocation at 36 weeks of gestation showing the left macular tumor on ultrasonography and (**C**) fundus photography. **D-F.** Corresponding imaging of the right eye showing a tiny posterior pole lesion sparing the fovea detectable only post-natally on both ultrasound and fundus photography (infero-temporal to the fovea). (Images courtesy of: Dr. Ants Toi, Medical Imaging, Mt. Sinai Hospital and Dr. Brenda Gallie, Ophthalmology and Vision Sciences. Hospital for Sick Children. University of Toronto. Canada).

Figure 7. Origin of retinoblastoma from maturing L/M cone precursors. The scheme depicts the generation of L/M cone precursors from a retinal progenitor cell, the increased expression of proliferation-related proteins during cone precursor maturation, the terminal differentiation of L/M cone photoreceptor, and the proposed stages of pRB-deficient cone precursor tumorigenesis. Recent studies indicate 1) that L/M-cone precursor maturation is associated with increased expression of pRB, MDM2, MYCN, and additional cell cycle proteins, 2) that pRB-

deficient cone precursors that have reached maturation stage marked by cone arrestin (ARR3) expression proliferate and avoid apoptosis under the influence of intrinsically highly expressed MYCN and MDM2, 3) that the majority of the initially proliferating pRB-deficient cone precursors enter a senescence-like retinoma state that rare cells escape or bypass to form retinoblastoma tumors. The maturing human cone precursor circuitry is thought to drive the expression of oncoproteins that sensitize to pRB loss, such as by RXR γ -dependent expression of MDM2 and MDM2-dependent expression of MYCN, which have so far been characterized in retinoblastoma cells (arrows). The model is based on data of Lee et al, 2006; Dimaras et al, 2008; Xu et al, 2009 and 2014; Qi et al, 2014; and Singh et al 2016 and Singh et al, 2018b.

Figure 8. A. Schematic timing of the first mutation in retinoblastoma. **a.** Inherited heritable **b.** Isolated heritable. **c.** Mosaic **d.** Non-heritable. The timing of the first mutation determines both the presence of a constitutional tumor predisposition and the risk of transmission to offspring.

In familial retinoblastoma (**a**) an oncogenic variant allele (rb) is inherited from a parent. The individual is heterozygous (RB rb) and half of the individual's gametes will carry the variant allele. Inactivation of the other allele in the retinoblast by a second mutation (red arrow) will trigger the development of retinoblastoma.

In isolated heritable retinoblastoma (**b**) the first mutation has occurred de novo prior to conception (pre-zygotic mutation). As the individual is heterozygous (RB rb) the situation regarding tumor predisposition and transmission to offspring is as in familial disease.

A first post-zygotic mutation may result in somatic mosaicism (**c**). The extension of the mutant sector and the risk of transmission to offspring depend on the timing and targeted cell type of this mutation. Risk of retinoblastoma oncogenesis is proportional to the extent of mutated retina.

Likewise, the proportion of gametes carrying the mutant allele is determined by the contribution of the mutant sector to the individual's germline.

A somatic first mutation in a retinoblast (d) is expected to predispose to one single tumor focus with no possible transmission to offspring (isolated non-heritable retinoblastoma).

B. Pedigrees /modes of presentation of retinoblastoma. **a.** Multigenerational, complete penetrance (bilateral retinoblastoma) **b.** Multigenerational, incomplete penetrance (uni/bilateral and unaffected) **c.** Two-generational, founder unilateral **d.** Isolated bilateral **e.** Isolated unilateral.

Figure 9. Retinoblastoma growth patterns. **A.** Endophytic retinoblastoma with class 1 (dust) and 2 (spheres) vitreous seeding. **B.** Exophytic retinoblastoma with peritumoral retinal detachment **C.** Exophytic growth with total retinal detachment causing complete spontaneous vitrectomy (kissing retinal bubbles). **D.** Mixed, predominantly exophytic growth with extended class 2 subretinal seeding and class 3 (cloud) vitreous seeding. **E.** Cavitory retinoblastoma with exophytic growth and coexisting endophytic retinoma before (**E**) and after (**F**) intra-arterial chemotherapy. **G.** Diffuse infiltrating retinoblastoma in a 14-year-old boy. White arrowheads delineating the posterior front line of the tumor as documented by OCT before (**H, H'**) and 3 weeks after (**I, I'**) the first course of systemic chemotherapy. The white asterisk in H shows the posterior limit of the tumor front line.

NB: Lines on fundus photograph correspond to the adjacent ultrasonography image or optical coherence tomography scan.

Figure 10. Primary aqueous humor invasion mechanisms in anterior diffuse retinoblastoma. A-B. Schematic illustration of anterior diffuse growth. In this case, the tumor presumably arises from the peripheral retina where it can grow both trans-orally directly into the posterior chamber (Petit's canal) and/or into the vitreous through the fenestrated limiting

membrane, as illustrated in (C) by fundus photography and UBM (50 MHz) of a primary oral tumor in an 11-year-old patient. **D-F**. Anterior diffuse growth with aqueous seeding (no retinal tumor) arising from ectopic ciliary retina (**E**) or from an oral retinal tooth (**F**).

NB: For the microanatomy of the schematic en-face view and longitudinal sections of the fundus extremus please refer to the legends given in Figure 3. Tumor and tumor spread into the different compartments are characterized by the yellow mass and yellow arrows respectively. Invaded aqueous and vitreous compartments are indicated in light yellow.

Figure 11. Schematic representation and peripheral clinical limits of intravitreal, retrohyaloid and subretinal seeding in endophytic and exophytic retinoblastoma growth patterns. A-C. Vitreous seeding from endophytic growing peripheral retinal tumor secondary to the rupture of the internal limiting membrane and hyaloid. **C**. Fundus picture and UBM (35 MHz) showing vitreous seeding respecting the anterior hyaloid with a tumor-free posterior chamber. **D-F**. Retrohyaloid class 3 seeding from an endophytic growing tumor, secondary to internal limiting membrane rupture but with an intact hyaloid (**D**, **E**). In this context. (**F**), anterior seeding extension is limited by the vitreous base, posteriorly to the ora serrata (**F**). **G-H**. Subretinal seeding from an exophytic growing tumor. (**I**) Fundus photography showing subretinal seeding reaching the ora serrata by gravity.

NB: For the microanatomy of the schematic en-face view and longitudinal sections please refer to the legends given in Figure 3. Tumor and tumor spread into the different compartments are characterized by the yellow mass and arrows respectively.

Figure 12. Head-position-dependent seeding location in supine position. A-C. Free moving class 3 vitreous seeding (cloud) revealing intact optic nerve head and macula. **D-F**. Displacement of class 2 retro-hyaloid pavementous seeding from temporal to nasal side of the retina by tilting

the head from right (**J**) to left (**K**). **G-K**. Free moving class 3 retro-hyaloid seeding (cloud) over the posterior pole (**F**) in primary position and with the head tilted to the right (**G,H**) and left (**J,K**). **L-K**. Displacement (**L, M**) of class 2 intra-cavitary seed with optical coherence tomography localization (**N**). **L.K**. Intra-cavitary class 2 seed displacement, with (**N**) optical coherence tomography confirming the intra-cavitary localization of the moving seed.

Figure 13. Aqueous seeding. (**A**) Class 1, (**B, C**) 2 and (**D**) 3 seeding. **E**. Anterior segment optical coherence tomography showing presence of hyper-reflective material over the corneal endothelium, iris and angle.

Figure 14. Invasion of the anterior uvea and aqueous seeding class 3. **A, B**. Iris and intra-ciliary body invasion documented by slit lamp photography and both longitudinal and transverse ultrasound biomicroscopy (35 MHz) in a 26-month-old boy with previous enucleation of the contralateral eye, before (**A**) and after (**B**) successful treatment. **C**. Fundus of the same patient at 18-month event-free follow-up (Snellen visual acuity 0.5).

Figure 15. Secondary aqueous humor invasion mechanisms: schematic representation (left column) and histopathologic correlation (right column). (NB the trans-oral or epiciliary pathway is illustrated in Figure 10 A-C).

A-B. Trans-ciliary pathway. Aqueous humor invasion secondary to choroido-ciliary growth into (**A**) the posterior chamber across the bi-layered ciliary epithelium and/or into the anterior chamber through the iris root. (**B**) Histopathology (HE 13x) showing invasion of the corpus ciliaris and infiltration of the iris root (inferior inset HE 63x) with disruption (asterix) of the ciliary epithelium (superior inset HE 126x). **C-D. Supra-ciliary pathway.** Aqueous invasion secondary to choroidal growth (**C**) spreading through the supra-ciliary space with infiltration of the iris root. (**D**) Histopathology (HE 24x) showing massive supra-ciliary tumor invasion with

anterior displacement of the ciliary body. **E-H Trans-hyaloid pathway (E)** Vitreous seeds anchored on the anterior hyaloid with trans-hyaloid passage. **(F)** Histopathology (HE 24x) with retro-hyaloid seeding crossing into the posterior chamber (Inset HE 252x). **(G)** Parietal tumor growing over the pars plana infiltrating the anterior hyaloid. **(H)** Histopathology (HE 13x) with infiltrated retina up to the ora and passage into the posterior chamber through the hyaloid.

Figure 16. Retinoblastoma invasion of the posterior chamber before (left column) and after (right column) successful treatment, documented by photography and longitudinal and transverse UBM (ultrasound biomicroscopy) (35 MHz). **A, B.** Invasion of Hannover's canal with class 2 seeds, treated with intracameral chemotherapy and anterior segment protontherapy. **C, D.** Invasion of Petit's canal, treated with intracameral chemotherapy. **E, F.** Involvement of the pars plicata, treated by intracameral chemotherapy and brachytherapy **G, H.** Involvement of the pars plicata and ciliary body, treated by intracameral chemotherapy and brachytherapy.

Figure 17. Pre- and post-regression growth patterns of retinoma. **A.** Endophytic sporadic unilateral retinoma displaying a type III regression pattern with inactive spontaneously regressed past vitreous seeding (insert: OCT showing hyperreflective class 2 vitreous seeds) in a 45-year-old asymptomatic man. **B.** Exophytic familial unilateral retinoma displaying a type III regression pattern with spontaneously regressed subretinal seeding and well demarcated past exudative retinal detachment in a 31-years-old asymptomatic woman. **C-E.** Fundus photograph, fluorescein and indocyanin green angiography in a case of spontaneous malignant transformation of a sporadic unilateral retinoma in a 34-year-old woman.

Figure 18: Schematic representation of chemotherapy exposure in aqueous and vitreous humor and plasma after different routes of drug delivery in several animal models.

Abbreviations: IV, intravenous infusion; IVi, intravitreal injection; IAC, intra-arterial chemotherapy; PO, periocular injection; C_{max}, maximum concentration; AUC, area under the concentration versus time profile; vit, vitreous humor; pl, plasma; aq, aqueous humor.

^aDose :0.1 mg (Nemeth et al. 2011); ^bDose:70 mg/kg (Laurie et al. 2005); ^cDose:2.4 mg (Simpson et al. 2002); ^dDose:18.7 mg/kg (Hayden et al. 2004); ^eDose: 10 mg (Mendelsohn et al. 1998); ^fDose: 18.7 mg/kg (Mendelsohn et al. 1998); ^gDose :10 µg (Nemeth et al. 2011); ^hDose: 2 mg/kg (Laurie et al. 2005); ⁱDose: 1 mg (Schaiquevich et al. 2012a); ^jDose: 4 mg (Taich et al. 2016); ^kDose: 1 mg (Schaiquevich et al. 2014); ^lDose: 1 mg (Carcaboso et al. 2007); ^mDose: 1 mg (Carcaboso et al. 2007); ⁿDose: 5µg (Buitrago et al. 2010); ^oDose :7 mg (Schaiquevich et al. 2012b); ^pDose: 15 µg (Buitrago et al. 2016); ^qDose: 0.4 mg/kg (Daniels et al. 2018)

Figure 19. Melphalan concentration versus time profile in (●, green) vitreous humor after 15 µg IVi (intravitreal) to rabbits, (■, blue) aqueous humor after 15 µg IVi to rabbits, (◆, black) retina concentrations after 15 µg IVi to rabbits, and (▲, red) vitreous humor after IAC (intra-arterial chemotherapy) of 7 mg to pigs. Dotted line represents the IC₅₀ (concentration of chemotherapy that reduces 50% the cell viability) determined in a commercial cell line of retinoblastoma (Schaiquevich et al. 2012b; Winter et al. 2016). (Data corresponds to results published in Schaiquevich et al. 2012b; Buitrago et al. 2016)

Figure 20. Total topotecan concentration versus time profile in (●, green) vitreous humor after 5 µg IVi (intravitreal) to rabbits, (▼, red) aqueous humor after 5 µg IVi to rabbits, (■, blue) vitreous humor after 1 mg IAC (intra-arterial) to pigs, (◆, yellow) vitreous humor after 1 mg PO (periocular) to rabbits, (▲, violet) vitreous humor after 1 mg IV (intravenous) to rabbits, (◆, black) retina concentration after 4 mg IAC to pigs, and (●) optic nerve concentrations after 4 mg IAC to pigs. Dotted line represents the IC₅₀ (concentration of chemotherapy that reduces 50%

the cell viability) determined in a commercial cell line of retinoblastoma (Laurie et al. 2005; Schaiquevich et al. 2012a; Winter et al. 2016). (Data corresponds to results published in Carcaboso et al. 2007; Schaiquevich et al. 2012a; Buitrago et al. 2010)

Figure 21. Five-year Kaplan-Meier survival curves for retinoblastoma IIRC group A-C eyes initially treated in Lausanne before and after 2008.

Figure 22. Five-year Kaplan-Meier survival curve for retinoblastoma IIRC group D and E eyes initially treated in Lausanne before and after 2008.

Figure 23. Changes in tumor thickness following systemic chemotherapy. Box and whisker plot shows the percentage change in tumor thickness following each systemic chemotherapy course (1, 2, 3, 4 and 5). The median change is given by the thick horizontal line that bisects each box. The waist of the box represents the interquartile range and the ends of the whiskers the 5 and 95 centiles. The number of eyes that underwent each course of chemotherapy is given in the corresponding upper part of the graphic. The repeatability limit of the ultrasound used (10 MHz detector) is approximately 0.3 mm. In this cohort, 90% of the tumors were greater than >3mm, and the repeatability limit corresponds to less than 10% of tumor thickness, as shown by the horizontal dotted line.

Figure 24. Influence of amblyopia treatment in fovea-involving retinoblastoma. Box and whisker plot shows the distribution of final visual acuity in three treatment groups. Group 1: only eye patients (permanent occlusion) Group 2: unilateral disease with active patching of unaffected eye. Group 3: unilateral disease with no patching. The median visual acuity of each group is denoted with a thick horizontal line that bisects the corresponding boxplot. The interquartile range is shown by the waist of the boxplot, when the waists of two boxplots do not overlap, this

represents a significant difference, which is denoted with an asterisk (* $p < 0.05$, *** $p < 0.001$).

The number of patient in each group is given on the top of the graphic.

Figure 25. Intravitreal melphalan-induced chorioretinopathy. A, B. Grade 2 chorioretinopathy involving ante-equatorial retina over two clock hours shown on fundus photography and fluorescein angiography. **C-E.** Fundus of a premature child born at 37 weeks of gestation by Caesarean section. **C.** Two weeks after birth, diagnosis of a small juxtapapillary group B familial retinoblastoma (white arrows) in the papillo-macular bundle of the left eye. **D.** Grade 4 retinopathy involving macular and peripapillary retina detected 1 week after the 3rd intravitreal melphalan injection of 14 μg performed elsewhere (axial length, 17 mm) induced by an unfortunate intravitreal injection in the still open Cloquet's canal (see F). **E.** At the age of 2 years 10 months, complete peripapillary and macular atrophy surrounded by a zone of partial atrophy (vision 20/400 with paracentral fixation at 6 years of age). **F.** Schematic representation of the intravitreal spaces and their relationship to the retina with retina in blue, internal limiting membrane in red and hyaloid in green: 1) bursa pre-macularis or posterior precortical vitreous pocket (virtual in the neonate). 2) Fovea. 3) Area of Martegiani. 4) Optic nerve head. 5) Connecting channel. 6) Cloquet's canal. 7) Ergellet's space. 8) Berger's space. 9) Ligament of Wieger. 10) Anterior hyaloid. 11) Posterior limit of the vitreous base. 12) Vitreous base with fenestrated internal limiting membrane. **G.** Grade 5 retinopathy with maculopathy and neuropathy after retro-hyaloid intravitreal injection performed elsewhere (Image courtesy of Dr Youssef) under a detached posterior hyaloid projecting anteriorly performed elsewhere as shown by the vertical axial ultrasonography (12MHz) (**H**). **I.** Schematic representation of H.

Figure 26. Intravitreal drug concentration with respect to axial length and injection dose of melphalan as calculated for an eye with completely regressed retinal tumor(s) (regression type IV), or 0% of vitreous volume occupied by tumor(s).

Figure 27. Choroidal occlusive vasculopathy. **A.** Grade 1a: sectorial incomplete sparing the fovea. Fundus showing nasal incomplete choroidal atrophy and preserved temporal choroid. **B.** Grade 3a: diffuse with <50% complete choroidal atrophy. **C.** Grade 4b: mixed diffuse with 50% complete atrophy involving the fovea and 50% incomplete choroidal atrophy. **D.** Grade 5a: diffuse >50% complete choroidal atrophy, but with spared fovea.

Figure 28. Example of a heat-activated derivative of melphalan that only becomes bioavailable at higher temperatures, allowing the thermoresponsive chain to be cut at the ester bond to release the drug.

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Table 1. Incidence rates of retinoblastoma in various age groups reported in the literature during the last 15 years

Age Group	Source	Region	Incidence/million (95% Confidence Interval)
Birth cohort	(Li et al., 2016)	Taiwan	58
	(Park et al., 2014)	Korea	53 (48-57)
	(Seregard et al., 2004)	Sweden and Finland	60 (54-66)
Live births	(Park et al., 2014)	Korea	59 (54-64)
	(Seregard et al., 2004)	Sweden and Finland	65 (60-71)
0-1 year	(MacCarthy et al., 2006)	Europe, ACCIS	20.4
0-4 years	(Fernandes, 2018)	United States, SEER	12.1 (11.3-13.0)
	(Park et al., 2014)	Korea	11.2 (10.2-12.2)
	(Rangamani et al., 2015)	India, 5 registries	9.6
	(MacCarthy et al., 2009)	Great Britain	10.0
	(Seregard et al., 2004)	Sweden and Finland	11.6 (10.7-12.5)
	(Khandekar et al., 2004)	Oman	8.3
	(MacCarthy et al., 2006)	Europe, ACCIS	7.5
0-5 years	(Jaradat et al., 2011)	Jordan	9.3
0-6 years	(Azar et al., 2006)	Australia, NSW	8
0-9 years	(Park et al., 2014)	Korea	5.3 (4.8-5.8)
5-9 years	(Fernandes, 2018)	United States, SEER	0.5 (0.4-0.6)
	(Rangamani et al., 2015)	India, 5 registries	2.0
	(MacCarthy et al., 2009)	Great Britain	0.4
	(MacCarthy et al., 2006)	Europe, ACCIS	0.5
0-14 years	(Rangamani et al., 2015)	India, 5 registries	3.5
	(Moreno et al., 2014)	Argentina	5.0 (3.5-6.4)
	(Gatta et al., 2012)	Europe, 64 registries	3.0 (2.7-3.3)
	(Khandekar et al., 2004)	Oman	4.0
10-14 years	(MacCarthy et al., 2006)	Europe, ACCIS	0.1

ACCIS = Automated Childhood Cancer Information System; NSW = New South Wales; SEER = Surveillance, Epidemiology, and End

Results program

Table 2. Estimated incidence of retinoblastoma in 2015 by world region*

	Population at Risk	Birth Rate/1,000	Estimated Annual Incidence	
			Median	95% Confidence Interval
Africa	1,194 million	33.6	2,293	2,060–2,526
Asia	4,420 million	16.5	4,258	3,824–4,691
Europe	741 million	10.5	464	417–511
Latin America	632 million	16.4	615	552–677
North America	356 million	12.4	264	238–292
World	7,383 million	18.6	7,983	7,170–8,795

* Based on total population, crude birth rate, and infant mortality rate as reported in the World Population Prospects: The 2017 Revision (United Nations, 2017), and the reported retinoblastoma incidence of 1 in 16,642 live births (95% confidence interval 15,105–18,528) by birth cohort analysis (Seregard et al., 2004), as described (Usmanov and Kivelä, 2014).

Table 3. TNMH classification (8th Edition)

Clinical Definitions (cTNM)		
Category	Subcategory	Description
cTX		Unknown evidence of intraocular tumor
cT0		No evidence of intraocular tumor
cT1		Intra-retinal tumor(s) with subretinal fluid ≤ 5 mm from base of any tumor
	cT1a	Tumors ≤ 3 mm and further than 1.5mm from disc and fovea
	cT1b	Tumors > 3 mm or closer than 1.5mm from disc or fovea
cT2		Intraocular tumor(s) with retinal detachment, vitreous seeding, or subretinal seeding
	cT2a	Subretinal fluid > 5 mm from the base of any tumor
	cT2b	Vitreous seeding and/or subretinal seeding
cT3		Advanced intraocular tumor(s)
	cT3a	Phthisis or pre-phthisis bulbi
	cT3b	Tumor invasion of choroid, pars plana, ciliary body, lens, zonules, iris, or anterior chamber
	cT3c	Raised intraocular pressure with neovascularization and/or buphthalmos
	cT3d	Hyphema and/or massive vitreous hemorrhage
	cT3e	Aseptic orbital cellulitis
cT4		Extraocular tumor(s) involving orbit, including optic nerve
	cT4a	Radiologic evidence of retrobulbar optic nerve involvement or thickening of optic nerve
	cT4b	Extraocular tumor clinically evident with proptosis and/or an orbital mass
cNX		Regional lymph nodes cannot be assessed
cN0		No regional lymph node involvement
cN1		Evidence of preauricular, submandibular, and cervical lymph node involvement
cM1		Distant metastasis without microscopic confirmation
	cM1a	Tumor(s) involving any distant site (e.g. bone marrow, liver) on clinical or radiologic tests
	cM1b	Tumor involving the CNS on radiologic imaging (not including trilateral retinoblastoma)
pM1		Distant metastasis with microscopic confirmation
	pM1a	Pathological evidence of tumor at any distant site (e.g. bone marrow, liver, or other)
	pM1b	Pathological evidence of tumor in the cerebrospinal fluid or CNS parenchyma
H		Hereditary Trait
	HX	Unknown or insufficient evidence of a constitutional RB1 gene mutation
	H0	Normal RB1 alleles in blood tested with demonstrated high-sensitivity assays
	H1	Bilateral retinoblastoma, retinoblastoma with an intracranial primitive neuroectodermal tumor (i.e., trilateral retinoblastoma), patient with family history of retinoblastoma, or molecular definition of a constitutional RB1 gene mutation

Pathological Definitions (pTNM)		
Category	Subcategory	Description
pTX		Unknown evidence of intraocular tumor
pT0		No evidence of intraocular tumor
pT1		Intraocular tumor(s) without any local invasion, or with focal choroidal invasion, or pre- or intralaminar involvement of the optic nerve
pT2		Intraocular tumor(s) with local invasion
	pT2a	Concomitant focal choroidal invasion and pre- or intralaminar involvement of the optic nerve head
	pT2b	Tumor invasion of stroma of iris and/or trabecular meshwork and/or Schlemm's canal
pT3		Intraocular tumor(s) with significant local invasion
	pT3a	Massive choroidal invasion (> 3 mm in largest diameter, or multiple foci of focal choroidal involvement totalling > 3 mm, or any full-thickness choroidal involvement)
	pT3b	Retrolaminar invasion of the optic nerve head, not involving the transected end of the optic nerve
	pT3c	Any partial-thickness involvement of the sclera within the inner two thirds
	pT3d	Full-thickness invasion into outer third of the sclera and/or invasion into or around emissary channels
cT4		Extraocular tumor(s) involving orbit, including optic nerve
	cT4a	Evidence of extraocular tumor: tumor at the transected end of the optic nerve, tumor in the meningeal spaces around the optic nerve, full thickness invasion of the sclera with invasion of the episclera, adjacent adipose tissue, extraocular muscle, bone, conjunctiva, or eyelids

Mallipatna, A.C et al. Retinoblastoma. In: Amin MB, Edge SB, Greene FL, Byrd DR, Brookland RK, Washington MK, et al., editors. AJCC Cancer Staging Manual, 8th ed. New York: Springer; 2017.0.819-31

Table 4. International Intraocular Retinoblastoma Classification (IIRC)

Group A	Small tumors (≤ 3 mm) confined to the retina (no seeding), at least 3mm from the fovea and 1.5mm from optic nerve.
Group B	Tumors (> 3 mm) confined to the retina in any location, with clear subretinal fluid ≤ 5 mm from the tumor margin
Group C	Localized vitreous and/or subretinal seeding (< 5 mm in total from tumor margin). If there is more than 1 site of subretinal/vitreous seeding, then the total of these sites must be < 6 mm. Up to 1 quadrant subretinal seeding may be present.
Group D	Diffuse vitreous and/or subretinal seeding (≥ 5 mm in total from tumor margin). Seeding more extensive than Group C. Retinal detachment > 1 quadrant
Group E	Massive retinoblastoma with anatomic or functional destruction of the eye with one or more of the following <ul style="list-style-type: none"> - Neovascular glaucoma - Massive intravitreal hemorrhage - Aseptic orbital cellulitis - Tumor anterior to anterior vitreous face - Tumor touching the lens - Diffuse infiltrating tumor - Phthisis or pre-phthisis

Linn Murphree A. Intraocular retinoblastoma: the case for a new group classification. Ophthalmol Clin North Am. 2005;18(1):41-54, viii

Table 5. International Retinoblastoma Staging System (IRSS).

Stage 0	Stage I	Stage II	Stage III: Regional extension	Stage IV: Metastatic disease	
Conservative treatment	Eye enucleated, Completely resected histologically	Eye enucleated, Microscopic residual tumor	A: overt orbital disease B: preauricular or cervical lymph node extension	A: Hematogenous metastases 1. Single lesion 2. Multiple lesions	B: CNS extension 1. Prechiasmatic lesion 2. CNS mass 3. Leptomeningeal disease

NB: In cases of bilateral disease, the staging depends on the eye with more advanced disease. Abbreviation: CNS=central nervous system
 Chantada, G et al. A proposal for an international retinoblastoma staging system. *Pediatric blood & cancer*. 2006;47(6):801-5.

Table 6. Retinoblastoma classification at relapse (RSU-Classification)

Category	Subcategory	Description
RX		Unknown evidence of retinal relapse
R0		No evidence of retinal relapse
R1		Intra-retinal relapse
	R1a	Localized (accessible to focal therapy including brachytherapy) retinal relapse at least > 3mm from the foveola and 1.5 mm from the optic nerve head
	R1b	Diffuse retinal relapse (any retinal relapse which is not localized) or any retinal relapse contiguous to the foveola (≤ 3 mm) or the optic nerve head (≤ 1.5 mm)
SX		Unknown evidence of seeding relapse
S0		No evidence of seeding relapse
S1		Subretinal seeding relapse
	S1a	Localized subretinal seeding (≤ 1 quadrant), at least > 3 mm from the foveola and 1.5 mm from the optic nerve head
	S1b	Diffuse (>1 quadrant) subretinal seeding or any subretinal seeding contiguous to the foveola (≤ 3 mm) and/or the optic nerve head (≤ 1.5 mm)
S2		Intravitreal seeding relapse
	S2a	Localized vitreous and/or retrohyaloid seeding (≤ 3 mm from retinal tumor),
	S2b	Diffuse vitreous and/or retrohyaloid seeding (any vitreous and/or retrohyaloid seeding which is not localized)
S3		Aqueous seeding relapse
UX		Unknown evidence of uveal relapse
U0		No uveal relapse
U1		Choroidal relapse
	U1a	Focal choroidal relapse (≤ 3 mm in largest diameter)
	U1b	Massive choroidal relapse (>3 mm in largest diameter)
U2 ^(x)		Intraciliary relapse (x = number of clock hours involved)
U3		Iris relapse

Table 7. Pseudoretinoblastoma: acquired and congenital diseases mimicking retinoblastoma.

<p>A. Developmental abnormalities</p> <ul style="list-style-type: none"> - Persistent hyperplastic primary vitreous (PHPV), persistent fetal vasculature syndrome - Posterior coloboma - Congenital retinal folds - Myelinated nerve fibers - Morning Glory Syndrome - Retinal dysplasia - Congenital cataract - Congenital retinoschisis - Incontinentia pigmenti - Norrie disease - Congenital retinal fold 	<p>C. Vascular abnormalities</p> <ul style="list-style-type: none"> - Coats' disease - Retinopathy of prematurity - Familial exudative vitreoretinopathy <p>D. Tumors.</p> <ul style="list-style-type: none"> - Retinal astrocytic hamartoma - Medulloepithelioma - Glioneuroma - Choroidal hemangioma - Retinal capillary hemangioma - Combined retinal hamartoma - Acute leukemia - Juvenile xanthogranuloma - Amelanic choroidal melanoma - Heterotopic brain tissue = retinal choristoma - Choroidal osteoma = choroidal choristoma
<p>B. Inflammatory & infectious disorders</p> <ul style="list-style-type: none"> - Ocular toxocariasis - Congenital toxoplasmosis - Congenital cytomegalovirus retinitis - Herpes simplex retinitis - Endogenous or metastatic endophthalmitis - Orbital cellulitis - Intraocular cysticercosis 	<p>E. Miscellaneous</p> <ul style="list-style-type: none"> - Rhegmatogenous retinal detachment - Vitreous haemorrhage - Trauma - Strabismus (Brückner's phenomenon) - Congenital corneal opacity

Table 8. Incidence of second primary neoplasms in different countries.

		Standardized incidence ratio (95 % Confidence interval)		Absolute excess risk of second primary tumors per 10 000 survivors per year
		Hereditary retinoblastoma survivors	Non-hereditary retinoblastoma survivors	
MacCarthy et al. (2013)	Great Britain	13.7 (11.3-16.5)	1.5 (0.9-2.3)	58
Kleinerman et al. (2005)	USA	19 (16-21)	1.2 (0.7-2.0)	97
Marees et al. (2008)	Holland	20.4 (15.6-26.1)	1.85 (0.96-3.24)	68

Table 9. Summary of the major studies on systemic chemotherapy as first line treatment for retinoblastoma

Author /year	N eyes/ patients	Drug regimen	Mean n Cycles (range)	Focal Treatments N=eyes (%)*	Globe salvage rate without EBR (%)			Overall globe salvage rate (%) (including EBR)	Mean follow-up (months) (range)
					Group I-III /A-C		Group IV-V/D-E		
Greenwald and Strauss, 1996	11/6	CE	6 (6-7)	11 (100%)	3 (75%)	RE : II=1/1 (100%) III=2/3 (67%)	3 (43%) RE : IV=1/1 (100%) V=2/6 (33%)	8 (73%) RE : II=1/1 (100%) III=3/3 (100%) IV=0/1 (0%) V=4/6 (67%)	26 (12-40)
Murphree et al., 1996	35/na	CEV	3 (1-6)	35 (100%)	10 (100%)	RE : I=3/3 (100%) II=7/7 (100%)	5 (20%) RE : IV=1/4 (25%) V=4/21 (19%)	15 (43%) RE : I=3/3 (100%) II=7/7 (100%) IV=1/4 (25%) V=4/21 (19%)	na
Gallie et al., 1996	28/21	CEV+ CsA	6 (2-12)	40 (100%)	15 (100%)	RE : I=6/6 (100%) II=5/5 (100%) III=4/4 (100%)	11 (85%) RE : IV=1/1 (100%) V=10/12 (83%)	26 (93%) RE : I=6/6 (100%) II=5/5 (100%) III=4/4 (100%) IV=1/1 (100%) V=10/12 (83%)	3 (1-57)
Beck et al., 2000	33/24	CE	3 (1-5)	33 (100%)	18 (100%)	RE : I=5/5 (100%) II=10/10 (100%) III=3/3 (100%)	2 (13%) RE : IV=0/1 (0%) V=2/14 (14%)	29 (88%) RE : I=5/5 (100%) II=10/10 (100%) III=3/3 (100%) IV=1/1 (100%) V=10/14 (71%)	31 (4-41)
Gombos et al., 2002	42/36	CEV	6	0 (0%)	22 (69%)	RE : I=1/4 (25%) II=17/19 (89%) III=4/9 (44%)	2 (20%) RE : IV=0/2 (0%) V=2/8 (25%)	34 (81%) ⁺ RE : I=4/4 (100%) II=17/19 (89%) III=7/9 (78%) IV=1/2 (50%) V=5/8 (63%)	33 (12-44)
Brichard et al., 2002	20/12	CEV	6 (2-6)	15 (75%)	8 (100%)	RE : I=3/3 (100%) II=3/3 (100%) III=2/2 (100%)	8 (67%) RE : V=8/12 (67%)	16 (80%) ⁺⁺ RE : I=3/3 (100%) II=3/3 (100%) III=2/2 (100%) V=8/12 (67%)	22 (8-40)
Rodriguez-Galindo et al., 2003	43/25	CV	8 (6-8)	0 (0%)	15 (63%)	RE : I=6/7 (86%) II=7/12 (64%) III=2/5 (40%)	5 (26%) RE : IV=0/3 (0%) RE V=5/16 (31%)	30 (70%) RE : I=7/7 (100%) II=10/12 (83%) III=3/5 (60%) IV=0/3 (0%) V=10/16 (63%)	32 (10-65)
Gunduz et al., 2004	105/71	CEV	6 (2-9)	75 (71%)	24 (71%)	RE : I=4/4 (100%) II=16/23 (70%) III=4/7 (57%)	16 (23%) RE : IV=14/35 (40%) V=2/36 (6%)	73 (70%) RE : I=4/4 (100%) II=22/23 (97%) III=6/7 (86%) IV=28/35 (80%) V=13/36 (36%)	26 (6-49)
Schiavetti et al., 2005	58/46	CE	(4-8)	58 (100%)	26 (74%)	RE : I=9/10 (90%) II=11/16 (69%) III=6/9 (67%)	3 (13%) RE : IV=2/6 (33%) V=1/17 (6%)	37 (64%) RE : I=9/10 (90%) II=12/16 (75%) III=6/9 (67%)	53 (11-125)

[illegible]

Table 10. First-line retinoblastoma management in bilateral cases depending on the IIRC Classification

Disease in first eye	Treatment	Disease in second eye	Treatment
Group A and B macula/papilla spared	Laser photocoagulation Cryocoagulation Plaque	Group A and B macula/papilla spared	Laser photocoagulation Cryocoagulation Plaque
Group A and B macula/papilla spared	Laser photocoagulation Cryocoagulation Plaque	Group B macula/papilla involved Group C, D and selected E	IAC ± IViC
Groups B macula/papilla involved Group C, D and selected E	IAC±IViC or IVC	Group B macula/papilla involved Group C, D and selected E	IAC ± IViC or IVC

Legends: IIRC=International Classification System, Linn Murphree A. Intraocular retinoblastoma: the case for a new group classification. Ophthalmol Clin North Am. 2005;18(1):41-54, viii ; IAC=intra-arterial chemotherapy; IViC=intravitreal chemotherapy; IVC=intravenous chemotherapy

Table 11. Summary of the major studies on first line and salvage intra-arterial chemotherapy for retinoblastoma

Author (year)	Eyes/ Patients	1°=first line IAC 2°=salvage IAC n (%eyes)	Classification Group: n (%eyes)	Treatment Prior IAC, n (%eyes)	Drug used, n (%eyes)	Mean cycles (range)	Additional treatments post IAC,	Globe salvage, n (%group)	Mean/medi an follow- up, months (range)	Extraocular extension, n (%eyes)	Pineoblastoma, n (%patients)	Metastasis, n (%patients)	Deaths, n (%patients)
Abramson et al., 2012a	30/na	1°=30 (100)	COG: B=19 (63) C=11 (37)	None=30 (100)	M=30 (100) T=na (na)	na	Laser =15 (50%) cryo =1 (3%) cryo+ laser =3 (10%)	B=19 (100) C=10 (90)	16 (5-33)	0 (0)	0 (0)	0 (0)	0 (0)
Palioura et al., 2012	37/37	1°=27 (73)	COG; D=31 (84) [§] E=6 (16) [§]	None=27 (100)	M=na (na) [§] M+T=na (na) [§] M+C=na (na) [§] C=na (na) [§] C+T=na (na) [§] MTX=na (na) [§]	4 (2-7) [§]	Focal trt=17 (46%) [§] Plaque = 4 (11%) [§]	1°=24 (89)	21 (1-42) [§]	0 (0) [§]	1 (3) [§]	1 (3) [§]	0 (0) [§]
		2°=10 (27)		IVC=9 (90) EBRT=3 (30)				2°=8 (80)					
Thampi et al., 2013	12/10	1°=12 (100)	ICRB: B=3 (25) C=1 (8) D=6 (50) E=2 (17)	None=12 (100)	M=12 (100)	2 (1-5)	Focal trt=5(42%) EBRT = 1 (8%) IVC = 2 (16%) Local therapy =2 (17%)	1°=7 (58) B=3 (100) C=1 (100) D=2 (33) E=1 (50)	15 (1-29)	0 (0)	0 (0)	0 (0)	0 (0)
Venturi et al., 2013	39/36	1°=17 (44)	TNM: 1a=1 (6) 1b=1 (6) 2a=7 (41) 2b=4 (24) 3a=4 (24)	None=17 (100)	M=17 (100)	4 (1-9)	Focal trt= 11 (65) IVC=1 (6)	1°=9 (56) [±]	13 (1-27) ^{±§}	0 (0) [±]	0 (0) [±]	0 (0) [±]	0 (0) [±]
		2°=22 (56)	TNM: 1b=11 (50) 2a=1 (5) 2b=10 (45)	IVC=21 (95) EBRT=1 (5) Plaque=1 (5) IVitC= 1 (5)	M=22 (100)	4 (1-9)	Focal trt =18 (82)	2°=20 (91)					
Bracco et al., 2013	52/48	1°=22 (42)	IIRC: B=4(18) D=18(82)	None=22 (100)	M=22 (100)	3 (1-6)	IVC=2 (4%) [§] Cryo=13 (25%) [§] Laser ablation =13(25%) [§]	1°=9 (41)	37 (19-55)	0 (0) [±]	0 (0) [±]	0 (0) [±]	0 (0) [±]
		2°=30 (58)	A=5 (17) B=14 (47) C=4 (13) D=7 (23)	IVC=30 (100) EBRT=1 (3) Plaque=1 (3) IVitC=1 (3)	M = 30 (100)	4 (1-9)	IvitC =6 (12%) [§] POT=6 (12%) [§] Sr C = 6(12%) [§] Plaque = 3 (6%) [§]	2°=19 (63)					
Shields et al., 2014	70/70	1°=36	ICRB: B=1 (3) C=4 (11) D=17 (47) E=14 (39)	No=36 (100)	M=25 (70) M+T=7 (19) M+C=4 (11)	3 (1-6)	na	1°=26 (72) B=1 (100) C=4 (100) D=16 (94) E=5 (36)	19 (na) [§]	0 (0)	0 (0)	0 (0)	0 (0)

		2°=34	na	IVC=34 (100)	M=26 (76) M+T=7 (21) M+C=1 (3)	3 (1-7)	na	2°=21 (62)		0 (0)	0 (0)	0 (0)	0 (0)
Ong et al., 2015	17/12	1°=6 (35)	ICRB: B=1 (17) D=1 (17) E=4 (66)	None=0 (100)	M=6 (100)	4 (1-6)	IvitC = 3 (50) Focal trt=4 (67) EBRT=2 (33) IVC=2 (33)	1°=4 (67) B=1 (100) D=1 (100) E=2 (50)	26 (11-43)	0 (0)	0 (0)	0 (0)	0 (0)
		2°=11 (65)	B=2 (18) C=1 (9) E=8 (73)	IVC=11 (100) EBRT=4 (36) IVitC=3 (27)	M=11 (100)	3 (1-6)	EBRT=3 (27%) Focal trt =2 (18%) IVitC =2 (18%)	2°=5(45) B=1 (50) C=1 (100) E=3 (38)	19 (5-35)	0 (0)	0 (0)	3 (50) [§]	2 (33) [§]
Yannuzzi et al., 2015	77/72	1°=77 (100)	COG : C=2 (Cebulla et al.) D=52 (68) E=23 (30)	None=77 (100)	na	na	TTT = 45 (58%) Cryo = 34 (44%) EBRT = 1 (1.3%) Plaque = 12 (15.6%) POC = 3 (3.9%) IVitC = 7 (9.1%) IVC = 5 (6.5%)	1°=67 (87)	39 (9-104)	1 (Cebulla et al.)	0 (0)	3 (4)	0 (0)
Akyuz et al., 2015	56/46	1°=12 (21)	ICRB: C=4 (33) D=6 (50) E=2 (17)	None= 12 (100)	M=12 (100)	3 (1-5)	IVC = 1 (1%) [§] Local therapy = 1(1%) [§] Radiotherapy = 3(5%) [§]	1°=9 (75)	21 (1-27)	0 (0)	0 (0)	0 (0)	0 (0)
		2°=44 (79)	A=7 (16) B=6 (14) C=12 (27) D=13 (30) E=6 (13)	IVC=44 (100)	M=44 (100)	2 (1-7)		2°=28 (64)	12 (1-28)	1 (3) [§]	0 (0)	1 (3) [§]	2 (6) [§]
Abramson et al., 2016a	112/103	1°=54 (48)	COG: D=54 (100)	None=47 (87) Bridge IVC=7 (13)	M=31 [§] M+T=38 (34) [§] M+C=2 (2) [§] M+T+C=39 (35) [§] C+T=1 (1) [§] C=1 (1) [§]	4 (1-9) [§]	Laser+cryo=54 (100%) [§] POC = 3 (3%) [§] Plaque = 12 (11%) [§] IVC = 2 (2%) [§] EBRT = 2 (2%) [§]	1°=46 (85)	38 (2-110)	0 (0)	1 2	3 (6)	1 (2)
		2°=58 (52)	COG: D=58 (100)	IVC=51 (100) EBRT=15 (26) Plaque=4 (7) POC=9 (16) Cryo only=1 (Cebulla et al.)				2°=42 (72)	32 (2-96)	0 (0)	0 (0)	0 (0)	0 (0)
Shields et al., 2016a	66/66	1°=66 (100)	ICRB: B=3 (4) C=4 (6) D=36 (55) E=23 (35)	None=66 (100)	M=34 (52) M+T=32 (48)	3 (1-6)	IvitC=11 (17) Focal trt not mentioned	1°=48 (73) B=3 (100) C=4 (100) D=30 (83) E=11 (48)	na (6-65)	0 (0)	0 (0)	0 (0)	0 (0)
Tuncer et al., 2016	24/22	1°=24 (100)	ICRB: D=17 (71) E= 7 (29)	None=24 (100)	M=20 (84) M+T=2 (8) M+T+C=2 (8)	3 (2-5)	TTT=2 (8) Cryo=4 (17) Plaque=2 (8) IvitC=3 (13)	1°=16 (67) D=13 (76) E=3 (43)	29 (6-55)	0 (0)	0 (0)	0 (0)	0 (0)

Munier et al., 2017b	25/25	1°=25 (100)	IIRC: D=25(100)	None=25(100)	M=25 (100)	na	Cryo+ttt+Laser IvitC IAC=1 (4)	1°=25 (100)	42 (20-90)	0 (0)	0 (0)	0 (0)	0 (0)
Abramson et al., 2017a.	106/na	1°=106 (100)	COG: D=74 (70) COG:E=32(30)	None=106 (100)	na	na	Plaques, TTT, POC, cryo, IVitC given in some	5-year Kaplan-Meier estimates: 80%	28 (0.5-101)	na	na	3 (2)	na
Chen et al., 2017	107/73	1°=30 (28) 2°=77 (72)	IIRC: B=11 (10) [§] C=11 (10) [§] D=56 (53) [§] E=29 (27) [§]	None=30 (100) IVC=64 (83) Laser=12 (16) POC=1 (1)	M=40 (37) M+T=67 (63)	3 (2-5)	na	1°+2°=84 (79) B=11(100) [§] C=11 (100) [§] D=44 (79) [§] E=18 (62) [§]	14 (3-28)	na	na	na	na
Funes et al., 2018	97/81	1°=35 (36)	IIRC: B=5 (14) C=8 (23) D=22 (63)	IVC=12(34)	M-containing regimen=25 (71) C±T =10(29)	5 (1-8)	IvitC=1(3) EBRT=1(3)	1°=24 (69)	49 (12-72)	1 (1) [‡]	0 (0)	0 (0)	1 (1) [‡]
		2°=62 (64)	na	IVC=62 (100) EBRT=12 (20)	M-containing regimen=52 (84) C±T=10 (16)	4 (1-14)	IvitC=2(3) EBRT=6(10)	2°=42 (68)		1 (1) [‡]	0 (0)	0 (0)	1 (1) [‡]

Legends: M = melphalan; T = topotecan; C = carboplatin; MTX = methotrexate; na = not available; COG=Children's Oncology Group; ICRB=International Classification for Retinoblastoma; IIRC=International Intraocular Retinoblastoma Classification; TNM = tumor node metastasis staging, IVC=intravenous chemotherapy; IVitC = intravitreal chemotherapy; trt=treatment; cryo=cryocouagulation; TTT=thermotherapy; EBRT=external beam radiotherapy; POC=periocular chemotherapy; Sr C=subretinal carboplatin

[§]Outcome for primary and secondary IAC were not reported separately

[‡]1 patient was lost to follow-up

[§]There were 11 eyes in 6 patients that had secondary IAC. Three (3/6, 50%) patients developed systemic metastasis and 2 (2/6, 33%) died despite receiving chemotherapy.

[‡]There were 44 eyes in 34 patients that had secondary IAC. One (1/34, 3%) patient had positive optic nerve cut margin after enucleation and one (1/34, 3%) had systemic metastasis. Both patients (2/34, 6%) died despite receiving treatment.

[‡]=patient died of extraocular dissemination in the context of refusal of timely enucleation

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Table 14. Intravitreal melphalan-induced retinopathy. Results of a retrospective review including 90 consecutive eyes receiving their primary treatment for vitreous disease in Jules-Gonin Eye Hospital with intravitreal melphalan between 2008 and 2017. There were no significant differences between eyes suffering Grade 1 or 2 versus Grade 3 melphalan-induced retinopathy regarding the mean time to develop the complication, the total injected dosis until the first clinical signs of the retinopathy, or the injected concentration when the latter was evaluated taking into account the age-related vitreous volume only. On the contrary, when tumor volume was subtracted from the age-related vitreous volume, the toxic retinopathy was significantly related to the intravitreal drug concentration ($p=0.04$).

Classification	Number of eyes	Mean time to retinopathy from the 1st injection (months)	Total intravitreal melphalan dosis received at time of retinopathy (μg)	Mean intravitreal melphalan concentration ($\mu\text{g/ml}$)	
				Evaluated without considering the intraocular tumor volume	Evaluated considering the intraocular tumor volume.
Grade 0	53	-	-	6.0	6.6
Grade 1	15	0.65	44.4	5.6	6.3
Grade 2	19	0.63	59.4	6.6	7.4
Grade 3	3	0.53	30.0	7.5	10.6

Table 12. Vitreous seeds classification, response to intravitreal melphalan and clinicopathologic correlation.

Classification Active vitreous seeds	Type	Clinical features ¹	Response to intravitreal melphalan ²	Regression types ^{1,2}	Histopathologic features ³ Active vitreous seeds
Class 1	Dust	Loose cellular spread usually at the edge of a retinal tumor	2-3 weeks to regress, receives least drug (median 20µg)/injections (median 3)	100% Regression Type 0: Complete disappearance	Scattered macrophages, necrotic cells, and individuals viable tumor cells
Class 2	Sphere	<i>Two types:</i> - translucent - whitish center (necrosis) surrounded by translucent mono- or multi-layered tumor cells <i>Localization:</i> - Free floating spheres - Seaweed-like or oval shaped tumors anchored to the internal face of the posterior hyaloid	6-7 weeks to regress receives medium amount of drug (median 30µg)/injections (median 5)	90% Regression Type 0	Spherical clusters of viable cells in tight clusters. -translucent center: viable central cells indistinguishable from the remaining sphere. -white/yellow center: necrotic/apoptotic center cells with surrounding viable cells. May demonstrate calcification in surrounding macrophages
				10% Regression Type I: Refrigent /and or calcified vitreous seeding or/and Regression Type II: Amorphous often non-spherical inactive residues with or without pigment	
Class 3	Cloud	Dense, cumulus-like spread infiltrates	30-32 weeks to regress receives most drug (median 33µg)/injections (median 8)	55%: Regression Type 0	Mostly composed of necrotic material, few macrophages, and rare viable cells. Cream-colored clouds include few red blood cells. Often presence of open spheres of viable cells at the outer rim of a cloud.
				45%: Regression Type I Regression Type II Regression Type III: combination of Type I and II	

¹Munier FL. Classification and management of seeds in retinoblastoma. Ellsworth Lecture Ghent August 24th 2013. Ophthalmic Genet. 2014;35(4):193-207²Francis et al. The classification of vitreous seeds in retinoblastoma and response to intravitreal melphalan. Ophthalmology. 2015;122(6):1173-1179³Amram et al. Vitreous seeds in retinoblastoma: clinicopathologic classification and correlation. Ophthalmology. 2017;124(10):1540-1547

Table 13. Summary of the main studies on intravitreal chemotherapy for retinoblastoma.

Author (year)	Eyes/ Patients	Drug used, n (%eyes)	Dose (μ g), n (%eyes)	Mean number of injections (range)	Vitreous seed control, n (% eyes)	Recurrence, n (% of VS with control)	Globe salvage n (%eyes)	Median/mean follow-up, months (range)	Extraocular extension, n (%eyes)	Metastasis, n (% patients)	Deaths, n (% patients)
Munier et al., 2012a	23/23	M=23 (100)	M 20-30 =23(100)	M= 5 (2-12)	21 (91)	3 (14) [§]	20 (87)	22 (9-31)	0 (0)	0 (0)	0 (0)
Ghassemi et al., 2014	9/9	M=9 (100) T=9 (100)	M 40 = 9 (100) T 8- 20 = 9 (100)	M = 2 (1-3)	9 (100)	0 (0)	6 (67)	16 (7-25)	0 (0)	0 (0)	0 (0)
Francis et al., 2014	16/16	M=16 (100)	M 30 = 16 (100)	M = 7 (5-8)	15 (94)	1 (7)	14 (88)	5 (1-11)	0 (0)	0 (0)	0 (0)
Suzuki et al., 2015	239/na	M=239 (100)	M 8-24 =24 (100)	M = 4 (1-25)	163 (68)	31 (19)	132 (55)	118 (33-256)	1 (<1%)	11 (5) [§]	5 (2) [¥]
Ji et al., 2016	19/17	M=19 (100)	M 20 = 19 (100)	M = 6 (1-15)	16 (84)	0 (0)	14 (74)	26 (17-42)	0 (0)	0 (0)	0 (0)
Shields et al., 2016b	40/38	M=40 (100) T=11 (27)	M 20 = 18 (45) M 25 = 7 (18) M 30 = 14 (35) M 40 = 1 (3) T 20 = 10 (90) T 25 = 1 (10)	M = 4 (1-6) T = 3 (1-5)	40 (100)	1 (3) [±]	35 (88)	36 (6-88)	0 (0)	0 (0)	0 (0)
Berry et al., 2017a	28/25	M=28 (100)	M 20-40=28 (100)	M=3 (1-10)	28 (100)	0 (0)	19 (68)	33 (9-51)	0 (0)	0(0)	0 (0)
Kiratli et al., 2017	39/37	M= all	M20-30=19 (49) M>30-40=20 (51)	M=2 (1-5)	27 (69)		22 (56%)	12 (6-38)	0 (0)	0 (0)	0 (0)
Rao et al., 2018b	17/17	T=17(100)	T 30=17 (100)	T=3 (2-6)	17 (100)	0 (0)	16 (94%)	24 (15-34)	0 (0)	0 (0)	0 (0)

Legends: N=number; M=melphalan; T=topotecan; [§] one was true recurrence, 7 months after first course of 5 injections, 2 were iatrogenic nature from tumor apex rupture at plaque application; [§] 11 patients had distant metastasis or intracranial invasion (5 of them also suffered orbital recurrence), all of whom had high-risk pathological factors for metastasis but refused adjuvant chemotherapy. [±] vitreous relapse with concomitant retinal recurrence. [¥] One patient lost to follow-up before completion of adjuvant chemotherapy.

Table 15. Estimated intravitreal concentration of melphalan with respect to eye size (age) and tumor volume. Concentrations falling within the recommended safety limits are in green, those in blue are too low to reach efficacy, and those in red are outside the predetermined safety limits, exposing the patient to a high risk of retinal damage.

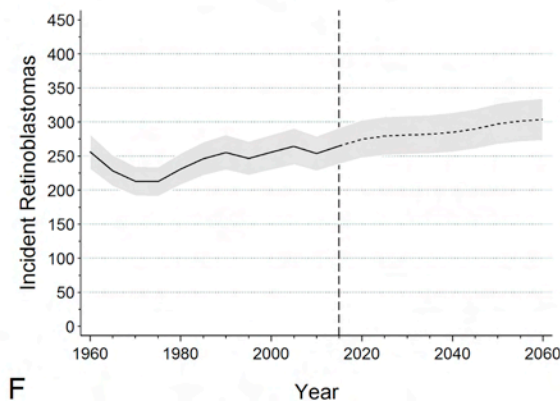
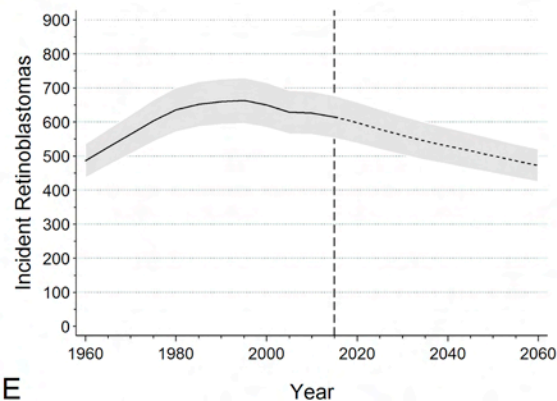
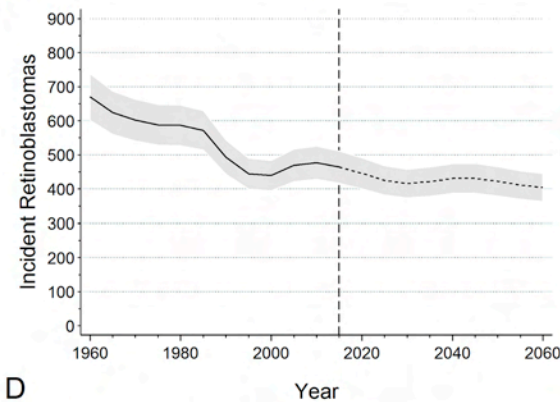
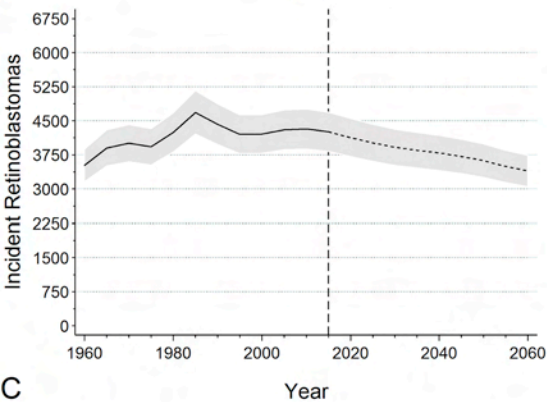
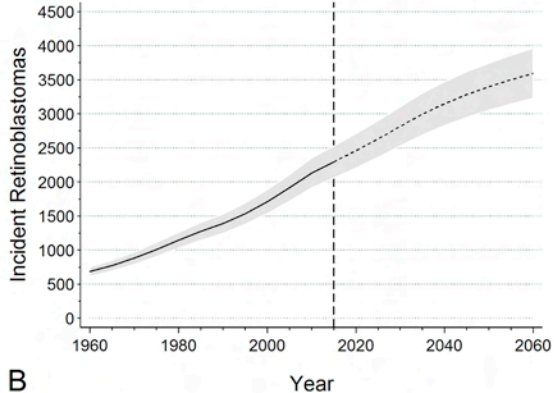
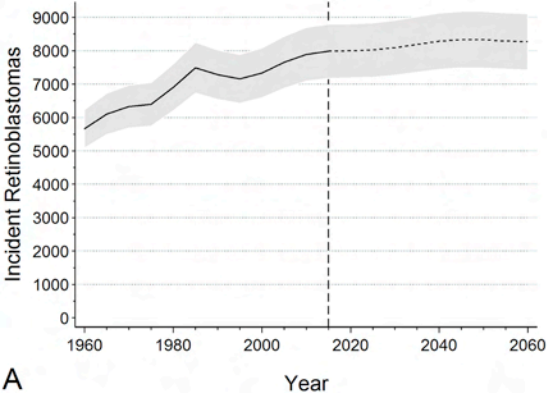
Tumor Volume	Dose	Vitreous concentration	
		At 6 months	At 36 months
0%	20µg	6µg/ml	4µg/ml
	30µg	9.5µg/ml	6.5µg/ml
	40µg	13µg/ml	8.2µg/ml
10%	20µg	7.5µg/ml	5µg/ml
	30µg	11µg/ml	7µg/ml
	40µg	14µg/ml	9µg/ml
25%	20µg	9µg/ml	5.5µg/ml
	30µg	13µg/ml	8µg/ml
	40µg	17µg/ml	11µg/ml
50%	12µg	7µg/ml	5µg/ml
	20µg	13µg/ml	8µg/ml
	30µg	19µg/ml	13µg/ml

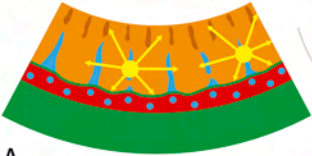
Table 16. Clinical classification of the choroidal occlusive vasculopathy after intra-arterial chemotherapy.

	Description
Grade 1	Any sectorial incomplete choroidopathy <ol style="list-style-type: none"> Sparing the fovea Not sparing the fovea Involvement of the fovea not assessable (due to tumor location or media opacities)
Grade 2	Any sectorial complete choroidopathy <ol style="list-style-type: none"> Sparing the fovea Not sparing the fovea Involvement of the fovea not assessable (due to tumor location or media opacities)
Grade 3	Any diffuse (=involving the whole fundus) choroidopathy with complete choroidal atrophy involving less than 50% of the fundus <ol style="list-style-type: none"> Sparing the fovea Not sparing the fovea Involvement of the fovea not assessable (due to tumor location or media opacities)
Grade 4	Mixed diffuse choroidopathy: fundus with 50% complete and 50% incomplete choroidal atrophy <ol style="list-style-type: none"> Complete atrophy sparing the fovea Complete atrophy not sparing the fovea Involvement of the fovea not assessable (due to tumor location or media opacities)
Grade 5	Any diffuse choroidopathy with complete choroidal atrophy involving more than 50% of the fundus <ol style="list-style-type: none"> Complete atrophy sparing the fovea Complete atrophy not sparing the fovea Involvement of the fovea not assessable (due to tumor location or media opacities)

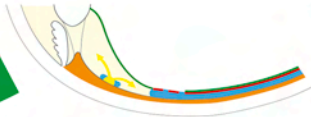
The authors have no financial interest in any aspect of this article.

No conflicting relationship exists for any author.

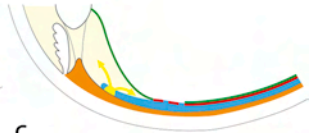




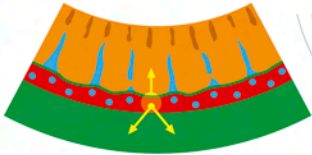
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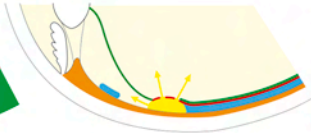
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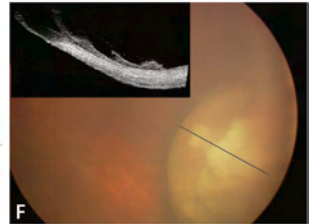
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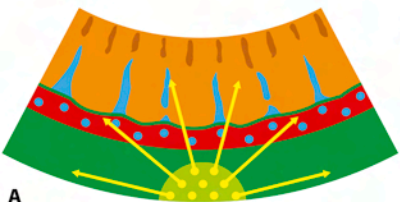
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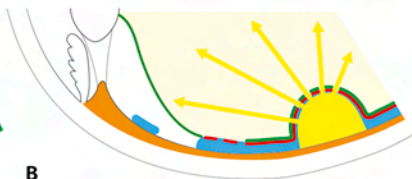
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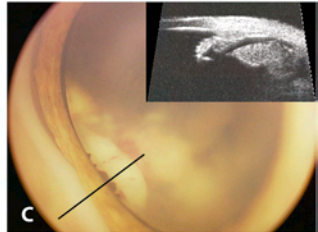
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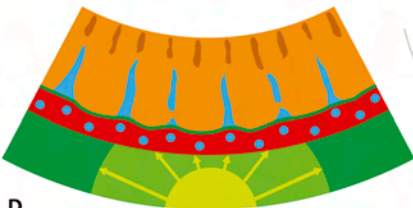
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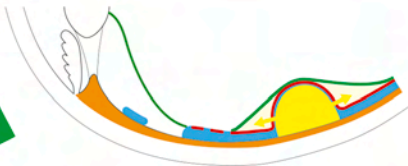
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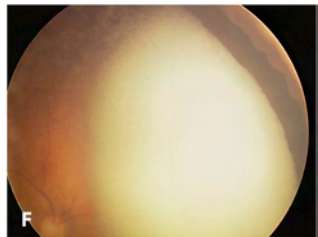
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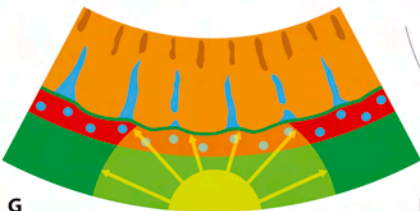
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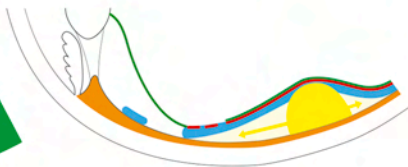
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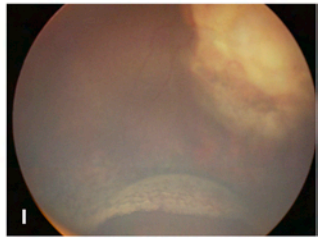
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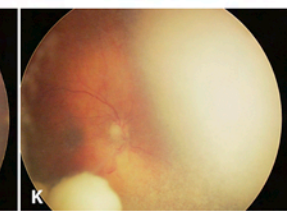
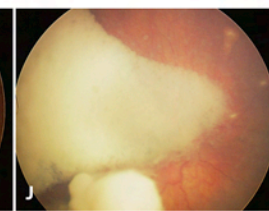
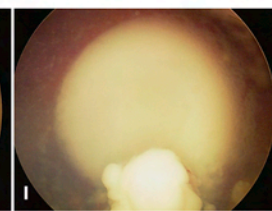
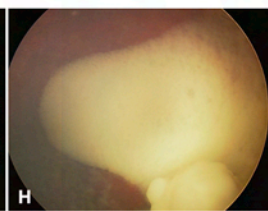
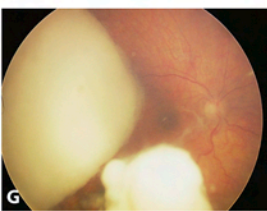
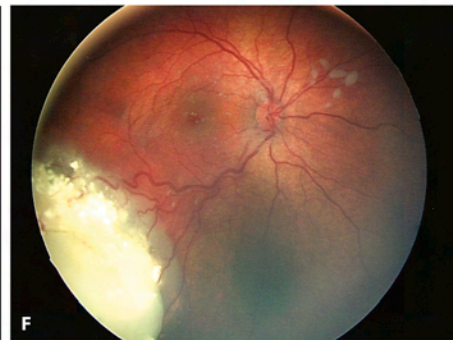
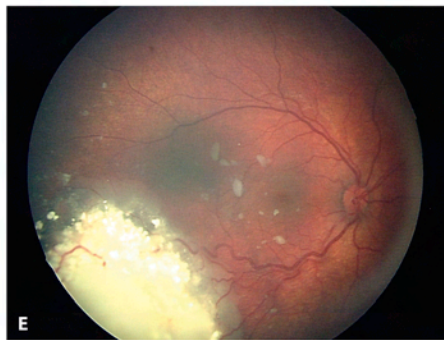
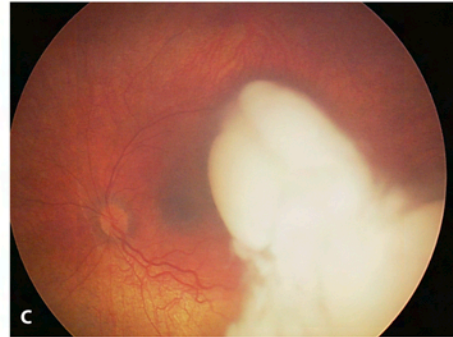
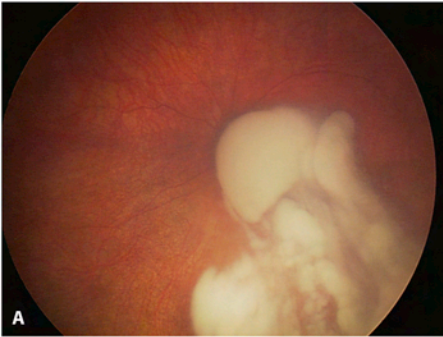
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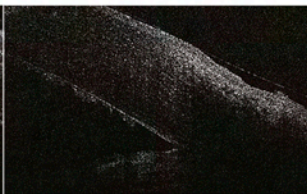
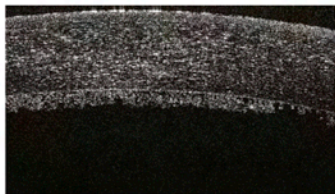
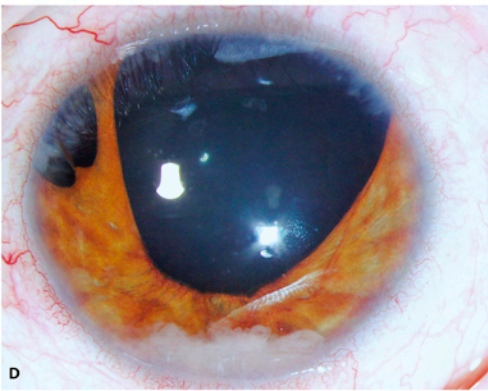
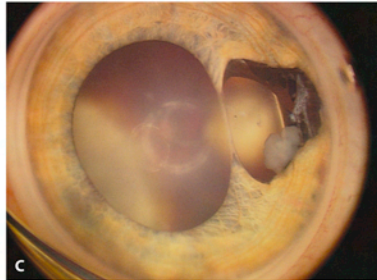
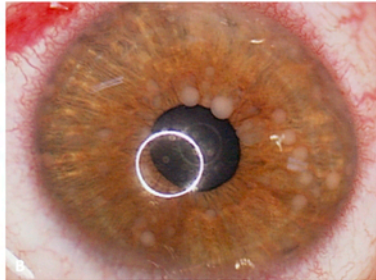
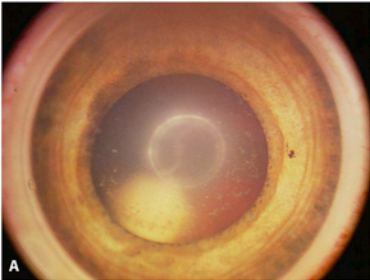


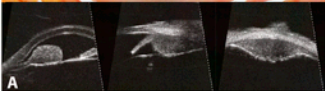
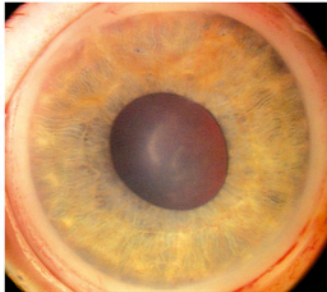
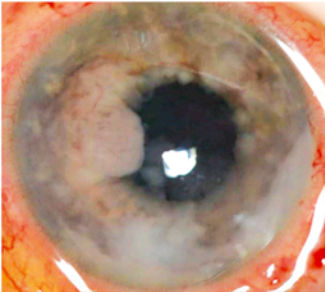
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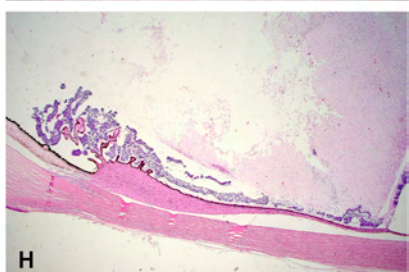
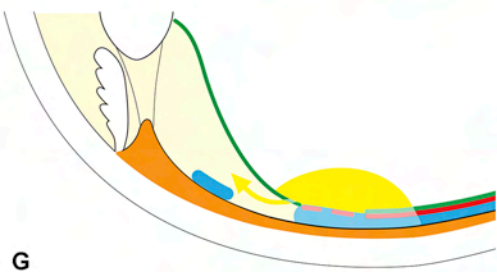
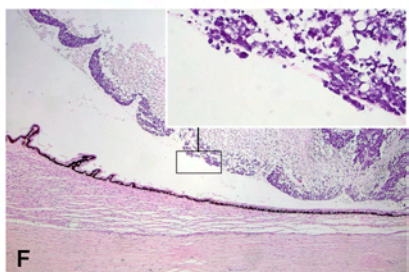
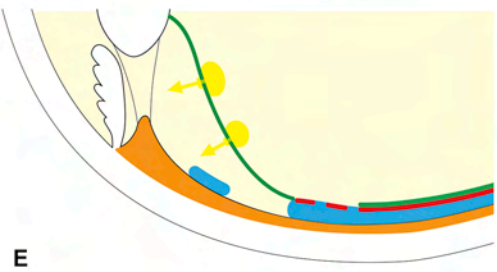
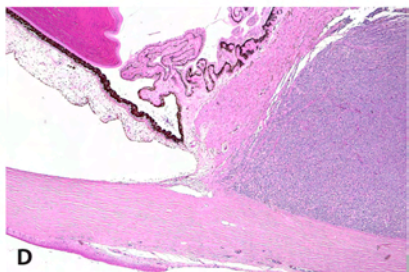
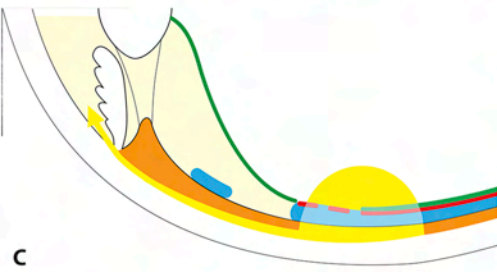
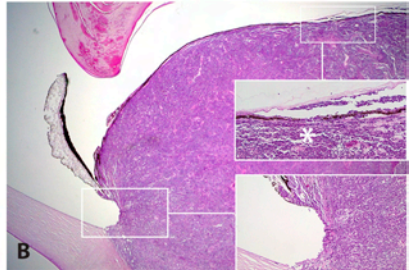
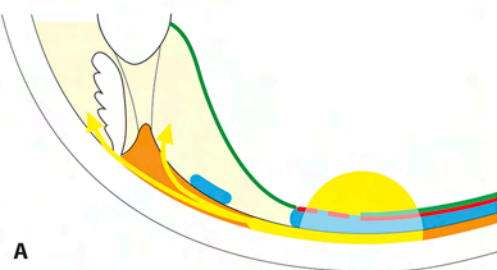


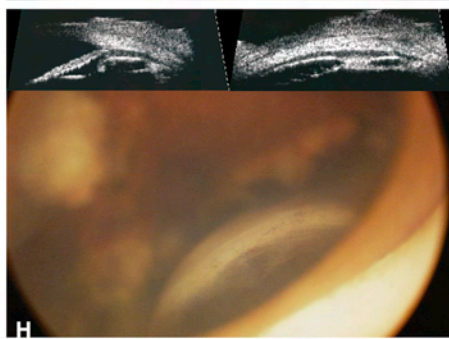
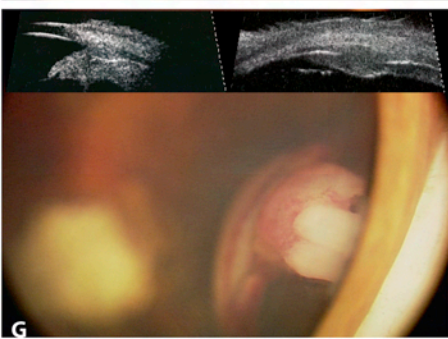
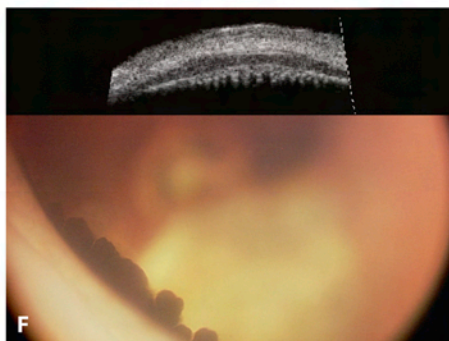
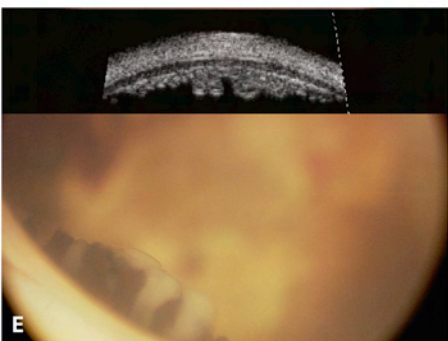
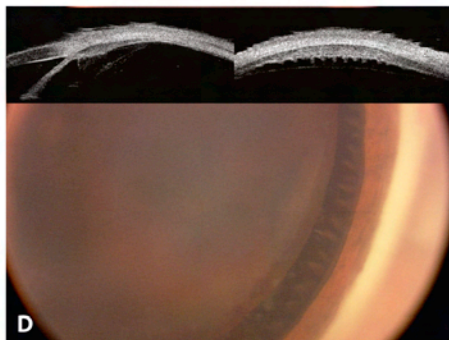
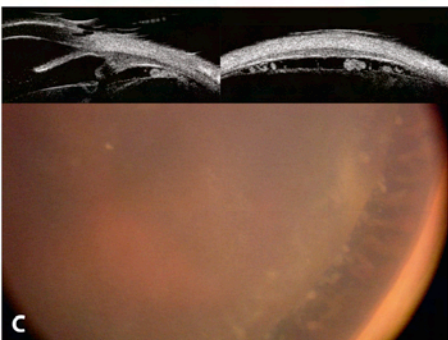
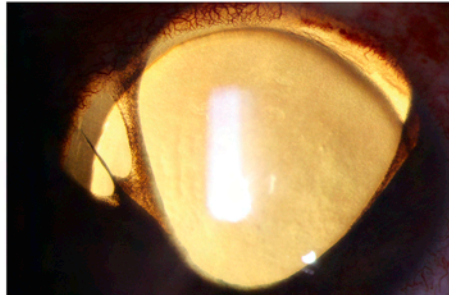
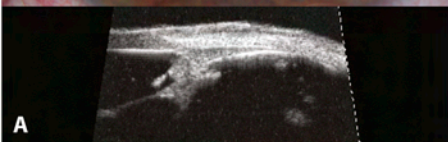
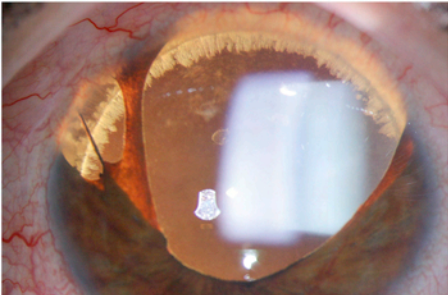
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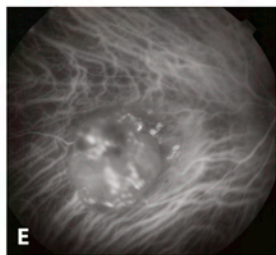
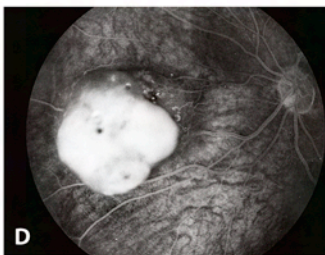
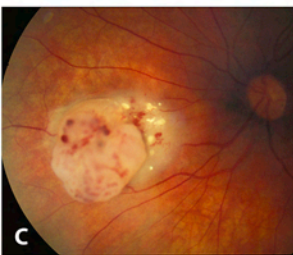
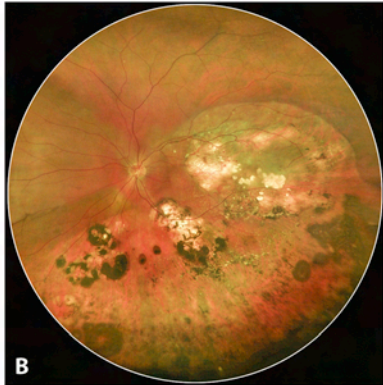
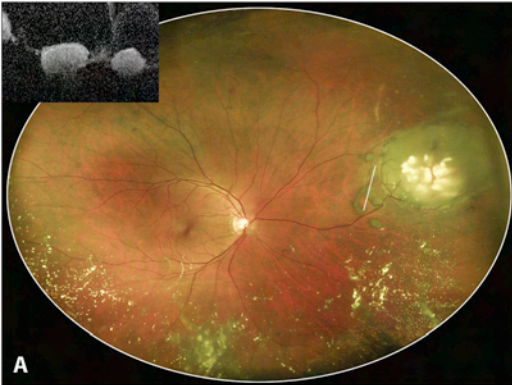












Carboplatin



PO -> AUC_{vit}/AUC_{pl} : 0.85 ^a

IV -> AUC_{vit}/AUC_{pl} : 0.59 ^b



PO -> $C_{max,vit}/C_{max,pl}$: 1.2 ^c

IV -> $C_{max,vit}/C_{max,pl}$: 0.2 ^d



PO -> $C_{max,vit}/C_{max,pl}$: 2.7 ^e

IV -> $C_{max,vit}/C_{max,pl}$: 0.01 ^f

Topotecan



PO -> AUC_{vit}/AUC_{pl} : 2 ^g

IV -> AUC_{vit}/AUC_{pl} : 0.38 ^h



PO -> AUC_{vit}/AUC_{pl} : 3.4 ⁱ

IV -> C_{vit}/C_{pl} : 0.09 ^j

IAC -> AUC_{vit}/AUC_{pl} : 29 ^k

PO -> AUC_{vit}/AUC_{pl} : 0.29 ^l

IV -> AUC_{vit}/AUC_{pl} : 0.23 ^m

IVi -> AUC_{vit}/AUC_{pl} : 57 ⁿ

Melphalan



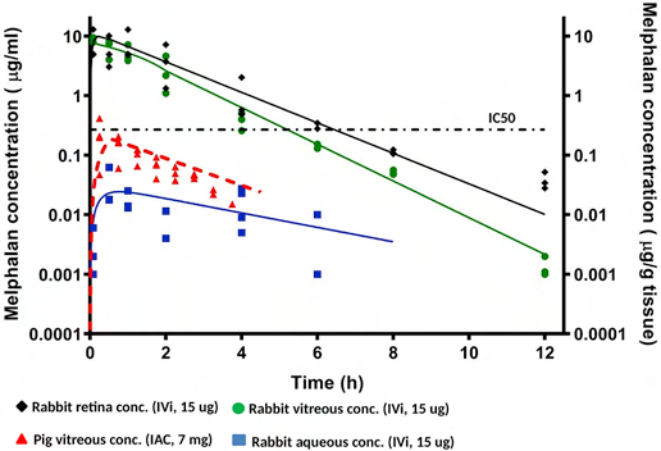
IAC -> AUC_{vit}/AUC_{pl} : 3 ^o

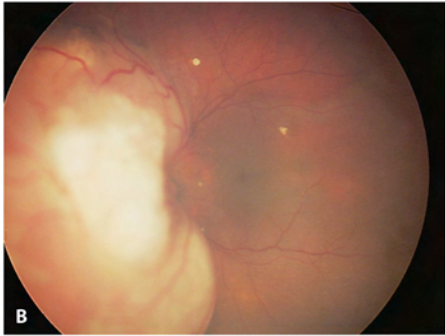


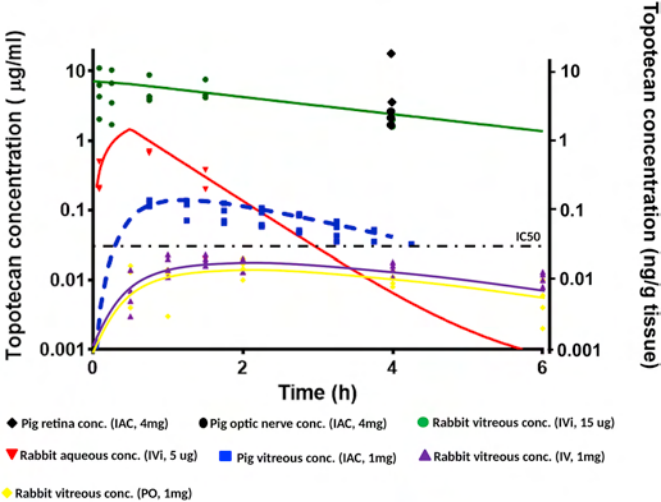
IVi -> $C_{max,vit}$: 7.8 ug/ml,

$AUC_{aq}/AUC_{vit} < 0.1$ ^p

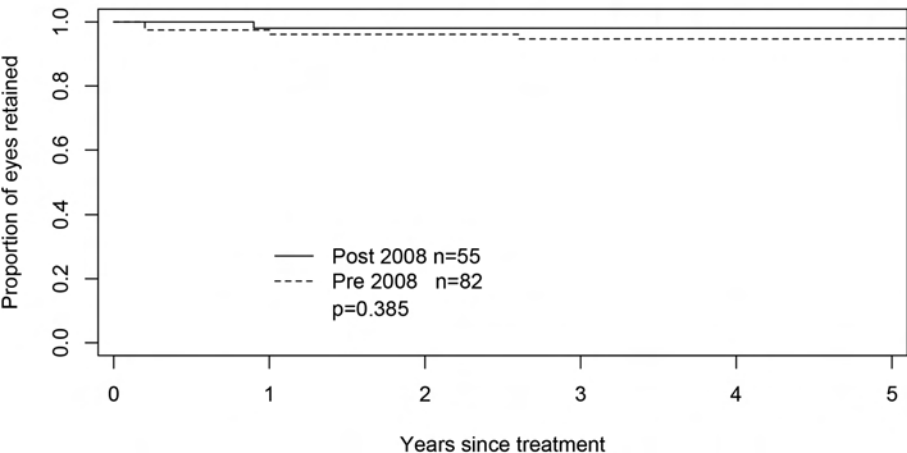
IAC -> AUC_{vit}/AUC_{pl} : 2 ^q



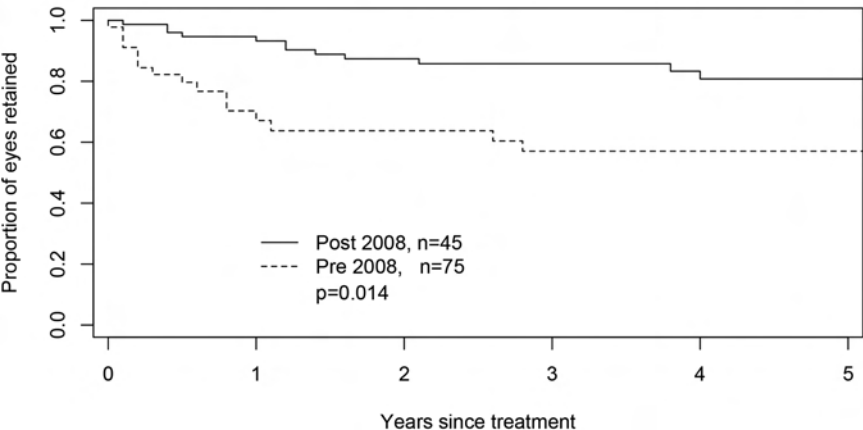




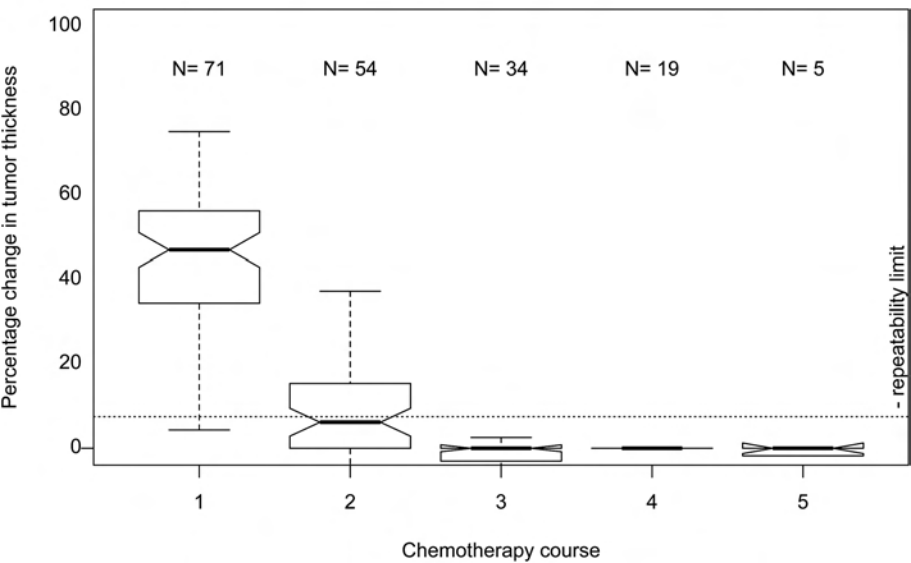
Kaplan-Meier for eye retention in Rb pre and post 2008 A-C eyes



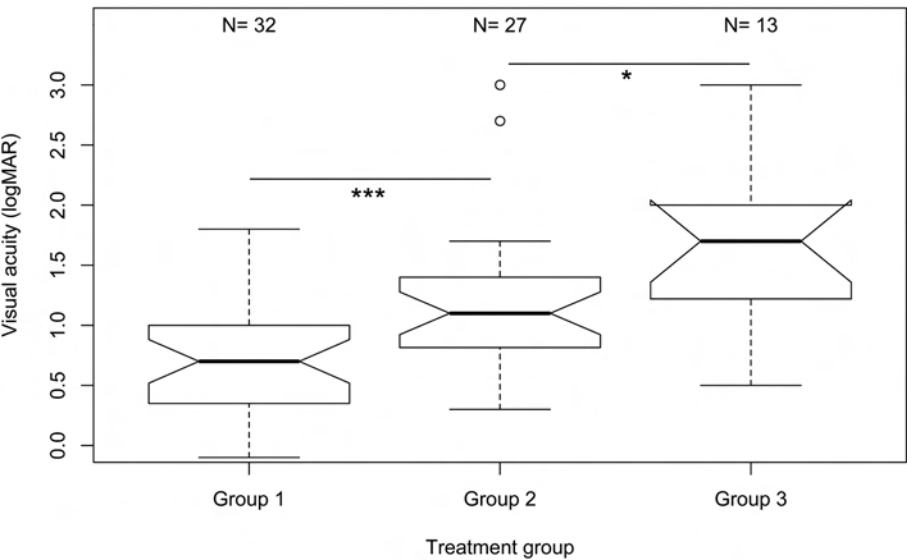
Kaplan-Meier for eye retention in Retinoblastoma pre and post 2008 D eyes

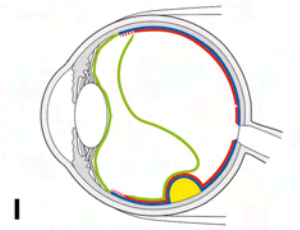
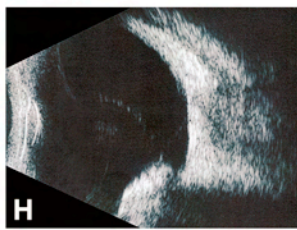
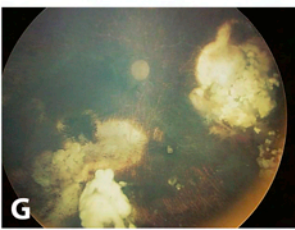
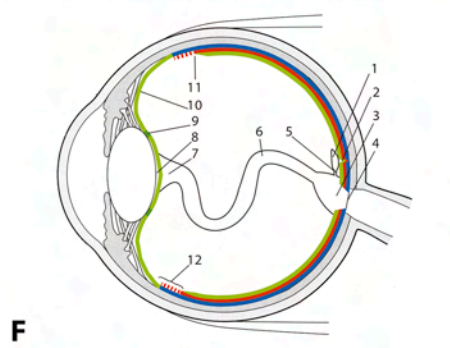
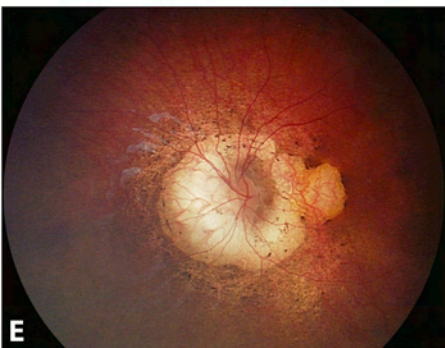
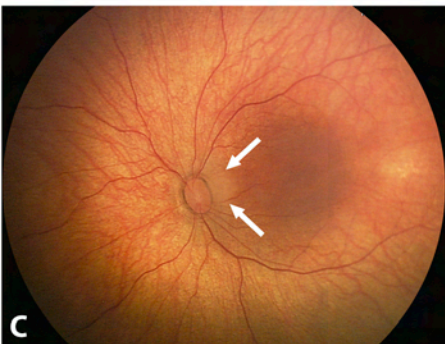
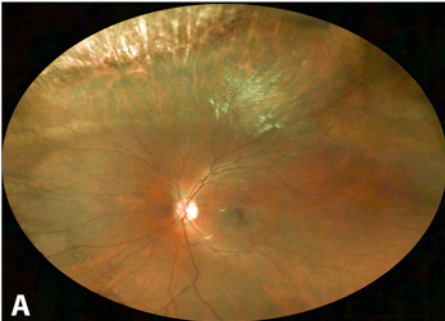


Percentage change in tumor thickness following chemotherapy



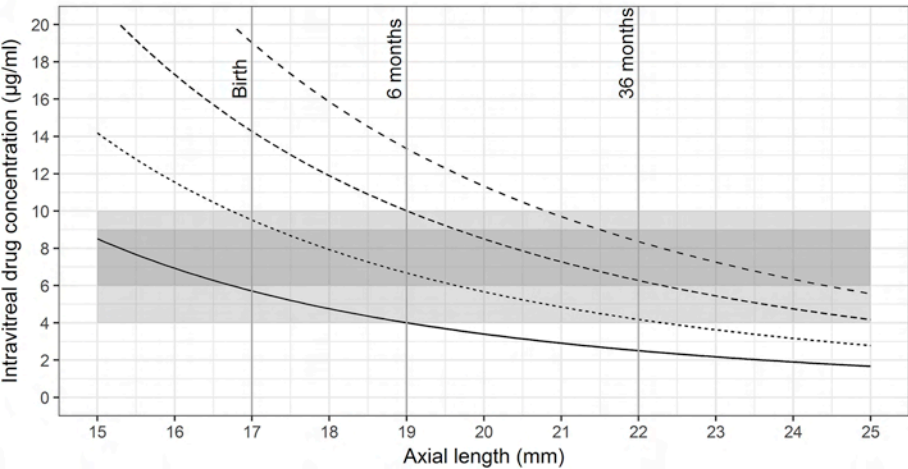
Visual acuity distribution by treatment group in macular retinoblastoma

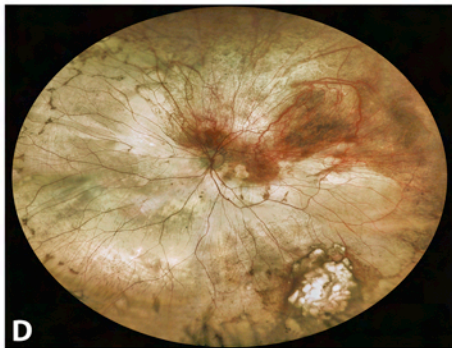
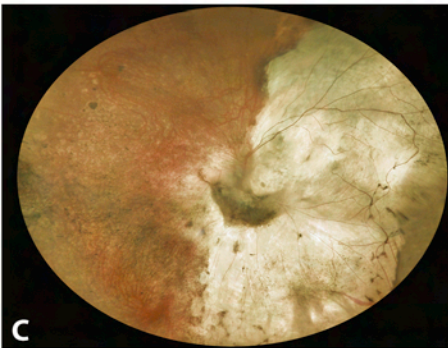
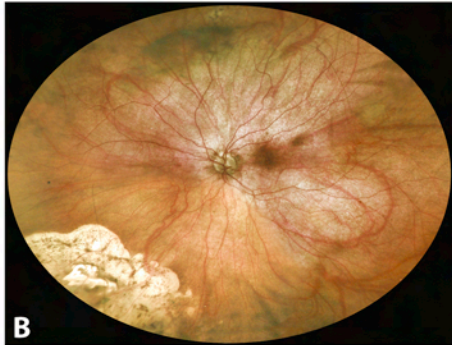
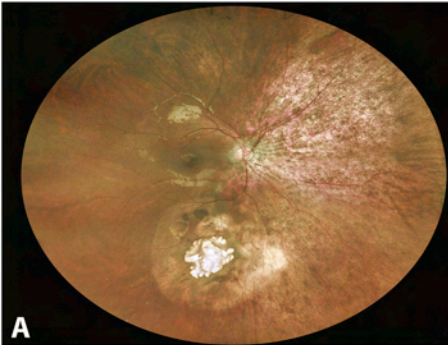


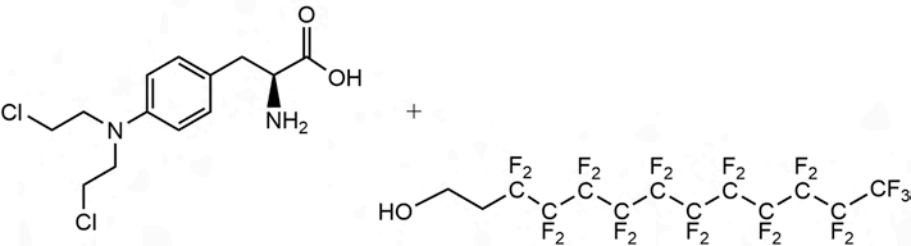
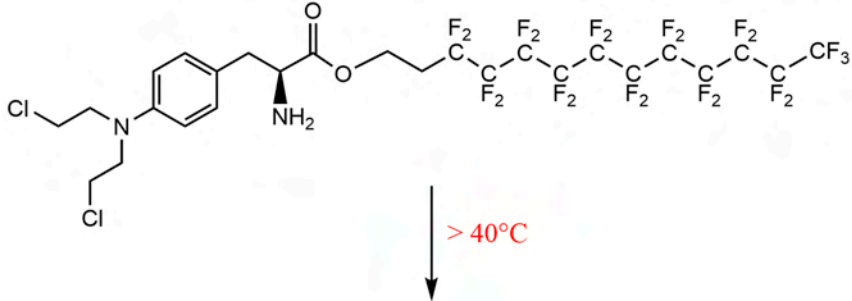


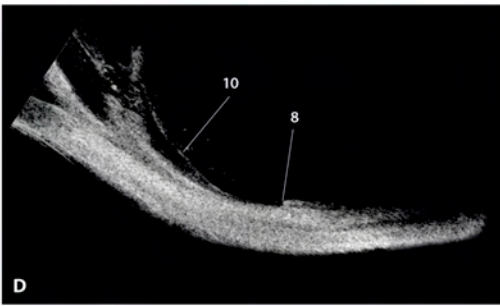
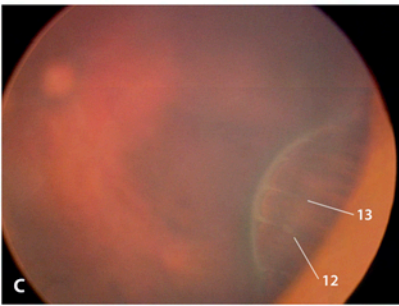
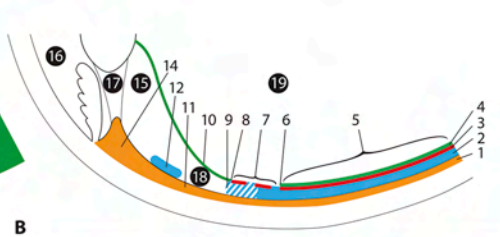
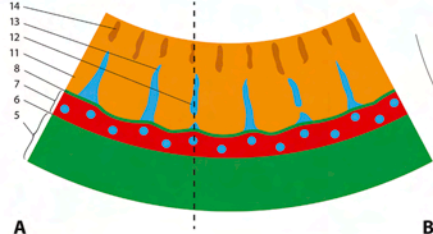
Intravitreal drug concentration with respect to axial length and injection dose

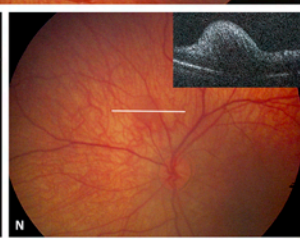
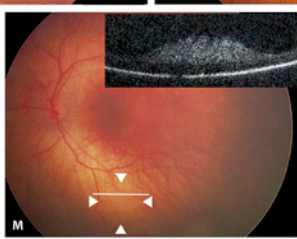
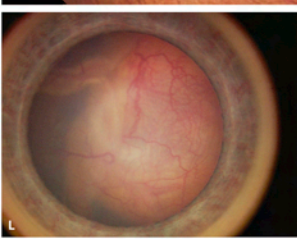
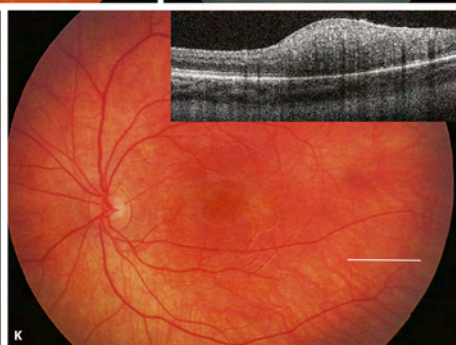
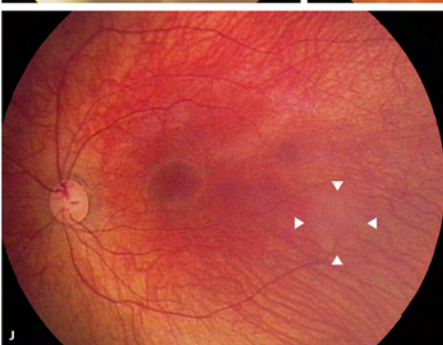
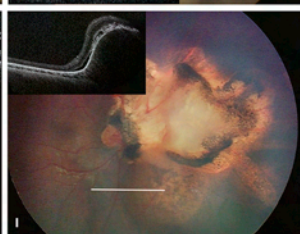
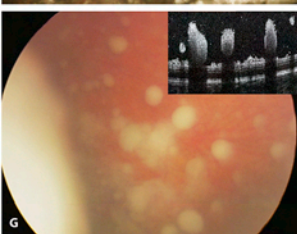
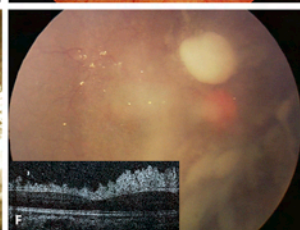
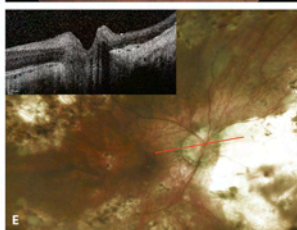
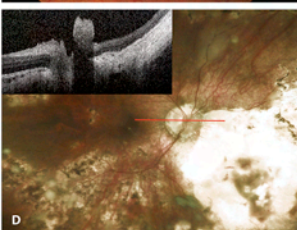
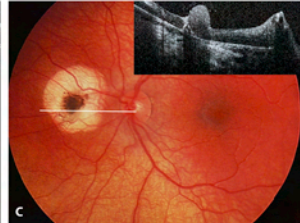
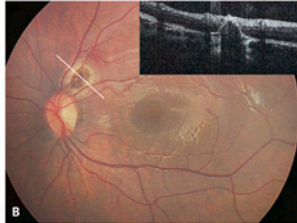
Injected dose: — 12 μ g 20 μ g --- 30 μ g -- 40 μ g

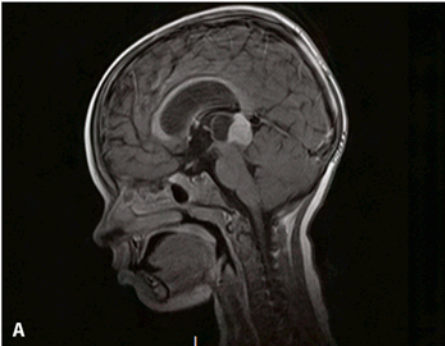




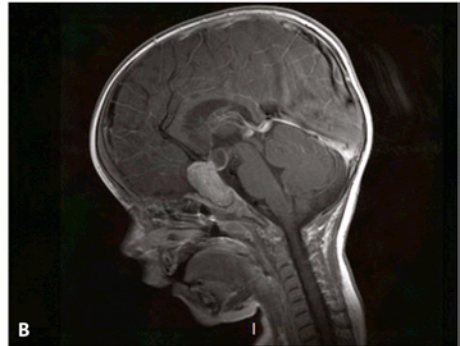








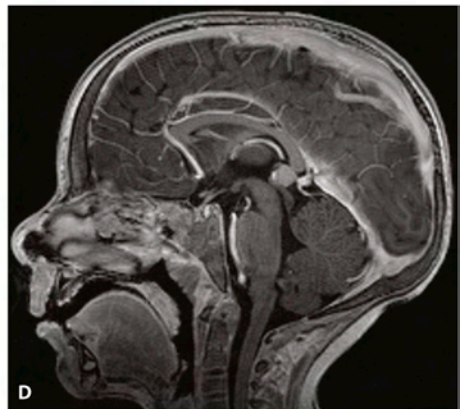
A



B



C



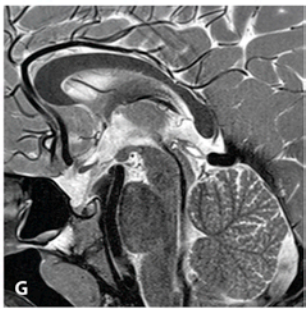
D



E



F

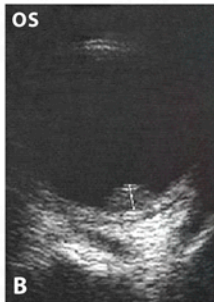


G

33w prenatal



36w postnatal



36w postnatal funduscopy

